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IN VITRO INHIBITION OF ACETYLCHOLINESTERASE IN THE HEAD AND BODY WALL OF *TRYPORYZA NIVELLA* (FABR.) FOLLOWING TREATMENT WITH ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES

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In vitro acetylcholinesterase (AChE) inhibition caused by six pesticides, viz., phorate, trichlorfon, diazinon, malathion, zectran and carbaryl in the head and body wall homogenate of sugarcane topshoot borer, *T. nivella* (F.) were investigated. All insecticides were found to be potent inhibitors of AChE and showed a dose and time dependent AChE blocking action. Similarly, in pI_{50} values indicate the presence of same AChE in both head and body wall although enzyme activity was about 25 times higher in the head. Relative AChE blocking activity has been explained on the basis of structure of pesticides. A tangible correlation between the LD_{50} and pI_{50} of these pesticides could not be seen.

(*Key words: Tryporyza nivella,* acetylcholinesterase, insecticides, inhibition)

INTRODUCTION

Organophosphorus (OP) and carbamate (Carb.) pesticides have insecticidal effect principally because of their ability to inhibit acetylcholinesterase (KOELLE, 1975) as a satisfactory correlation has been shown to exist between pest toxicity and AChE inhibition in a number of cases (MENGLE & CASIDA, 1960; O' BRIEN, 1967; METCALF, 1971; CORBETT, 1974; HUTACHARERN & KNOWLES, 1974). In the present study AChE inhibiting properties of certain selected pesticides were studied in the sugarcane topshoot borer, *T. nivella* (F.) larvae in an earlier study were found to be sensitive to OP and Carb. insecticides (PANDEY & AGARWAL, 1980).

MATERIALS AND METHODS

Bored shoots of sugarcane were collected from insecticide free-plots at the sugarcane research station Gorakhpur, U. P. Mixed groups of III and IV instar larvae of *T. nivella* (F.) were taken out from these, their head and body walls were dissected and used for biochemical studies.

Insecticides

Insecticides which were used for their biochemical studies were : zectran (4-dimethylamino-3, 5-xylyl methyl carbamate), carbaryl (1-naphthyl methyl carbamate), malathion [0, 0-dimethyl-S-1, 2-di (carboethoxy) ethylphosphorodithioate], phorate [0, 0-diethyl-S- (ethylthio) methyl phosphorodithioate], trichlorfon [0, 0-dimethyl-(1-hydroxy-2, 2, 2,-trichloroethyl) phosphonate], diazinon [0, 0-diethyl-0-(2-isopropyl-4-methyl-6-pyrimidyl)-phosphorothioate].

Acetylcholinesterase activity

The head (0.25% w/v) and bodywall (2.5% w/v) from five larvae were homogenized in 0.02 M cold phosphate buffer, pH 7.0 by a motor driven homogenizer for 5 min in an ice bath. The homogenates were centrifuged at 2000 g at -4 C. Supernatants were separated and used as enzyme source. The enzymatic hydrolysis of acetylcholine (ACh) was measured by the method of HESTRIN (1949) in which a mixture consisting

of 1.0 ml homogenate, 2.0 ml of buffer and 1.0 ml of 0.004 M ACh were incubated at 37°C for 15 min. The enzyme activity was expressed as μ mol ACh hydrolyzed/mg protein/15 min. Protein was estimated by the method of LOWRY *et al.* (1951).

Inhibition of AChE 'in vitro'

Solutions of OP and Carb. insecticides were prepared in acetone and were diluted to the desired strength in the same solvent. In order to study dose-response effects of insecticides on AChE inhibition requisite amounts of insecticide solutions were pipetted in test tubes and acetone was allowed to evaporate. Controls contained acetone alone. Five different doses of the inhibitors were incubated with enzyme for 60 min at 37°C prior to the addition of ACh (0.004 M). The quantity of ACh not hydrolyzed was proportional to the inhibited enzyme. Inhibition data have been expressed as PI_{50} (the negative logarithm of the molar inhibitor concentrations yielding 50% inhibition of enzyme) of different insecticides. For the time-course studies the enzyme was allowed to remain with insecticide for varying lengths of time i. e., 15, 30, 45 or 60 min prior to the addition of ACh (0.004 M). The amount of different inhibitors chosen for this study were selected from dose response data and were such which could evoke easily measurable activity even with low incubation periods.

All the experiments were replicated at least six times and data have been expressed as Mean ± SEM. Analysis of variance (SOKAL & ROHLF, 1969) and Student Newman Keul's test were used for evaluating significance.

RESULTS

Tables 1 and 2 show that the activity of AChE (mg/protein) in the head was far greater (approximately 25 times) as compared to the body wall. The effects of varying doses of OP viz., phorate, trichlorfon, diazinon and malathion and Carb. viz., zectran and carbaryl on the AChE activity in the head and body wall homogenates of *T. nivella* (F.) are given in Tables 1 and 2. It can be clearly seen from Tables 1 and 2 that the activity of AChE in head and bodywall homogenates was inhibited to varying degrees following 1 h exposure to OP and Carb. insecticides. Analysis of variance showed that the change of dose significantly altered ($P<0.01$) the inhibition of AChE activity both in head and bodywall. Tables 1 and 2 indicate that there was more than 91% inhibition of AChE in the head and more than 88% in bodywall following 1 h exposure to the highest doses of the six insecticides. Tables 1 and 2 also give the inhibitory potency (PI_{50}) values of the six pesticides. The order of inhibitory potency in the head was phorate > malathion > trichlorfon > zectran > diazinon > carbaryl and phorate > malathion > trichlorfon > diazinon > zectran > carbaryl in the bodywall.

Time-course studies:

Time-course studies on the inhibition of AChE activity following treatment with phorate (7.6×10^{-8} M), trichlorfon (1.9×10^{-8} M), diazinon (8.1×10^{-9} M), malathion (6.9×10^{-9} M), zectran (4.5×10^{-9} M) and carbaryl (2.9×10^{-8} M) for 15, 30, 45, or 60 min have been given in Table 3. Analysis of variance of data given in Table 3 show that except for inhibition by zectran of the bodywall AChE, the effect of insecticides was time-dependent Table 4, however, brings out the fact that the time taken for maximum inhibition varied from pesticide to pesticide. Thus maximum inhibition was achieved by zectran in 15 min whereas it took 60 min for trichlorfon for maximal inhibition of AChE in both tissues (Table 4).

Relationship between 'in vitro' AChE inhibition and toxicity:

Figure 1 shows that relationship between toxicity as represented by LD_{50} (μg a.i./larva) and *'in vitro'* anti-AChE activity of the six insecticides as reflected by the PI_{50} values of these. It can be seen that a tangible relationship does not

TABLE 1. *In vitro* inhibition of head AChE of *T. nivella* (F.) by various doses of differents insecticides.

Insecticide Dose (M a. i.[1])	Control	AChE : μ mol ACh hydrolyzed/mg protein/15 min Treated[1]					pI_{50}[2]
*Phorate** 3.8×10^{-9}, 7.6×10^{-9}, 2.3×10^{-8}, 3.8×10^{-8} or 5.7×10^{-8}	8.11±0.23 (100)	5.95±0.27+ (74.36)	3.47±0.48+ (52.00)	3.34±0.50+ (41.18)	2.65±0.26+ (32.67)	0.40±0.12+ (4.93)	7.84
*Trichlorfon**							
3.8×10^{-9}, 1.1×10^{-8}, 1.9×10^{-8} 5.8×10^{-8} or 9.7×10^{-8}	8.10±0.32 (100)	6.72±0.28 (86.96)	5.31±0.23+ (65.55)	4.00±0.18+ (49.38)	2.43±0.16+ (30.00)	0.47±0.15+ (5.71)	7.69
*Diazinon**							
3.2×10^{-9}, 8.1×10^{-9}, 2.4×10^{-8}, 4.0×10^{-8} or 4.9×10^{-8}	8.13±0.47 (100)	7.06±0.26 (86.83)	5.26±0.67+ (64.69)	4.18±0.52+ (51.50)	3.12±0.53+ (38.37)	0.65±0.06+ (7.99)	7.57
*Malathion**							
3.0×10^{-9}, 6.9×10^{-9}, 2.0×10^{-8}, 3.4×10^{-8} or 4.5×10^{-8}	8.19±0.14 (100)	7.59±0.11 (92.67)	4.11±0.55+ (50.18)	3.76±0.49+ (45.90)	3.25±0.29+ (39.68)	0.60±0.14+ (7.32)	7.32
*Zectran**							
4.5×10^{-9}, 1.3×10^{-8}, 2.2×10^{-8}, 3.1×10^{-8} or 4.0×10^{-8}	8.43±0.37 (100)	6.79±0.22+ (80.54)	5.80±0.36+ (68.80)	4.53±0.34+ (53.73)	3.50±0.32+ (41.51)	0.24±0.11+ (2.94)	7.6
*Carbaryl**							
9.9×10^{-9}, 2.9×10^{-8}, 4.9×10^{-8}, 7.4×10^{-8} or 1.2×10^{-7}	8.28±0.23 (100)	7.11±0.19+ (85.86)	5.36±0.32+ (64.73)	3.46±0.24+ (41.78)	1.70±0.23+ (20.53)	0.68±0.19+ (8.21)	7.33

Table showing AChE activity. Mean ± SEM following treatment with 5 different doses of six pesticides. Six replicates were done for each experiment. Values in parentheses indicate per cent AChE activity with untreated controls taken as 100%.

1. The data on treated tissue have been given in the same sequence in which doses have been indicated.
2. pI_{50} = negative logarithm molar inhibitor concentration for 50% inhibition.

* ($p < 0.01$) significant when analysis of variance was applied to see whether changes of dose significantly altered enzyme activity.

\+ ($p < 0.01$) significant when compared with control using Student's t test.

TABLE 2. *In vitro* inhibition of bodywall AChE of *T. nivella* (F.) by various doses of different insecticides

Insecticide Dose, (M a. i.[1])	AChE : μmol ACh hydrolyzed/mg protein/15 min Control	Treated[1]					pI_{50}[2]
*Phorate**							
3.8×10^{-9}, 7.6×10^{-9}, 2.3×10^{-8}, 3.8×10^{-8} or 5.7×10^{-8}	3.16±0.15 (100)	2.77±0.12 (88.49)	0.79±0.12+ (25.00)	0.65±0..0++ (20.50)	0.48±0.10++ (15.18)	0.19±0.06++ (6.01)	7.84
*Trichlorfon**							
3.8×10^{-9}, 1.1×10^{-8}, 1.9×10^{-8}, 5.8×10^{-8} or 9.7×10^{-8}	3.24±0.15 (100)	2.84±0.13+ (87.65)	2.18±0.12+ (67.28)	1.20±0.06+ (34.03)	0.56±0.15+ (17.28)	0.13±0.05+ (4.01)	7.69
*Diazinon**							
3.2×10^{-9}, 8.1×10^{-9}, 2.4×10^{-8}, 4.0×10^{-8} or 4.9×10^{-8}	3.22±0.03 (100)	2.93±0.09+ (90.91)	1.57±0.21+ (49.75)	1.36±0.18+ (42.23)	1.14±0.17+ (35.40)	0.25±0.05+ (7.76)	7.57
*Malathion**							
3.0×10^{-9}, 6.9×10^{-9}, 2.0×10^{-8}, 3.4×10^{-8} or 4.5×10^{-8}	4.19±0.04 (100)	3.00±0.07 (94.04)	2.00±0.28+ (62.69)	1.57±0.31+ (49.29)	1.49±0.32+ (46.70)	0.33±0..8+ (10.34)	7.72
*Zectran**							
4.5×10^{-9}, 1.3×10^{-8}, 2.2×10^{-8}, 3.1×10^{-8} or 4.0×10^{-8}	3.28±0.16 (100)	2.91±0.18 (88.71)	2.67±0.17++ (81.40)	2.19±0.21++ (66.76)	1.43±0.15+ (44.23)	0.17±0.04+ (5.18)	7.6
*Carbaryl**							
9.9×10^{-9}, 2.9×10^{-8}, 4.9×10^{-8}, 7.4×10^{-8} or 1.2×10^{-7}	3.19±0.16 (100)	2.76±0.12++ (86.52)	2.21±0.06+ (69.27)	1.49±0.13+ (46.63)	0.69±0.15+ (21.63)	0.36±0.08+ (11.28)	7.33

Table showin AChE activity Mean ± SEM following treatment with 5 different doses of six pesticides, viz., phorate, trichlorfon diazinon, malathion, zectran and carbaryl

++ ($p < 0.05$) Significant when compared with control using Student *t* test. Other signs and abbreviations are as in Table 1.

TABLE 3. *In vitro* inhibition of head and bodywall AChE of *T. nivella* (F.) following exposure to different insecticides for varying time periods.

Insecticide (Dose M a. i.)	AChE : μ mol ACh hydrolysed/mg protein/15 min					
	Phorate 7.6×10^{-9}	Trichlorfon 1.9×10^{-8}	Diazinon 8.1×10^{-9}	Malathion 6.9×10^{-9}	Zectran 4.5×10^{-9}	Carbaryl 2.9×10^{-5}
Head						
Control	8.41±0.43* (100)	8.21±0.23* (100)	8.21±0.40* (100)	8.43±0.49* (100)	8.34±0.13* (100)	8.22±0.25* (100)
Exposure time (min)						
15	8.03±0.41 (95.48)	7.92±0.21 (96.45)	7.36±0.36 (88.64)	7.74±0.34 (91.90)	7.80±0.19 (93.52)	7.75±0.26 (94.28)
30	6.94±0.34++ (83.52)	7.14±0.24+ (86.96)	5.57±0.67+ (67.84)	6.34±0.56++ (75.32)	7.50±0.23++ (88.92)	5.92±0.25+ (72.50)
45	3.85±0.30+ (45.77)	5.23±0.24+ (63.70)	5.01±0.46+ (61.02)	4.74±0.29+ (56.22)	7.04±0.27+ (84.41)	5.72±0.35+ (59.58)
60	3.47±0.48+ (41.26)	4.00±0.18+ (48.72)	5.26±0.67+ (64.06)	4.11±0.55+ (48.75)	6.79±0.22+ (81.41)	5.36±0.32+ (65.20)
Body wall						
Control	3.19±0.16* (100)	3.17±0.15* (100)	3.26±0.29* (100)	3.59±0.30** (100)	3.28±0.08 (100)	3.18±0.83* (100)
15	2.78±0.11++ (87.14)	3.09±0.16 (97.47)	2.93±0.36 (88.87)	3.44±0.36 (95.82)	3.11±0.06 (94.51)	2.93±0.11++ (92.13)
30	2.43±0.10++ (72.17)	2.59±0.15++ (81.70)	2.39±0.30++ (73.31)	2.41±0.31+ (67.13)	3.02±0.07 (92.07)	2.78±0.64+ (87.42)
45	1.01±0.11+ (32.60)	1.75±0.17+ (55.20)	1.81±0.18+ (55.52)	2.23±0.31+ (62.11)	2.87±0.08++ (87.50)	2.36±0.06+ (74.21)
60	0.79±0.12+ (24.77)	1.20±0.06 (37.85)	1.57±0.21+ (48.15)	2.00±0.28+ (35.71)	2.90±0.18++ (88.71)	2.21±0.06+ (69.49)

Table showing AChE inhibition Mean ± SEM following exposure for varying length of time to different pesticides. Six replicates were done for each treatment. Values in parentheses indicate per cent AChE activity with untreated controls taken as 100%.

* ($p < 0.01$); ** ($p < 0.05$) — Significant when analysis of variance was applied to see whether change in time significantly altered enzyme activity.

+ ($p < 0.01$; ++ $p < 0.05$)— Significant when compared with controls using Student *t* test.

TABLE 4. Time taken for maximum inhibition of AChE in head and bodywall following treatment with pesticides.

Insecticide	Exposure time for maximum effect (min)	
	Head	Bodywall
Phorate	45	45
Trichlorfon	60	60
Diazinon	30	15
Malathion	45	30
Zectran	15	15
Carbaryl	30	45

The time taken for maximum AChE inhibition following treatment with six pesticides. Student-Newman-Keul's test was applied between upper and lower intervals to see if significant change in inhibition took place with increase in time, based on data given in Table 3.

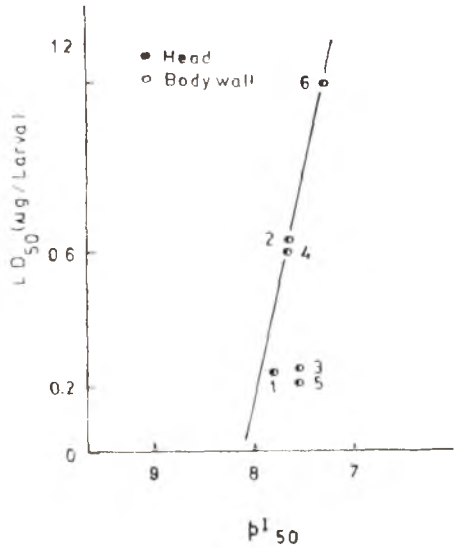

Fig. 1. Relationship between insecticidal toxicity (LD_{50}; Panday and Agarwal, 1980) and '*in vitro*' AChE inhibition (pI_{50}) of six insecticides in the head and bodywall homogenate of *T. nivella* (F).1. Phorate; 2. Trichlorfon, 3. Diazinon, 4. Malathion, 5. Zectran, 6. Carbaryl.

seem to exist between toxicity and AChE inhibition.

DISCUSSION

The results of the present study throw important light on the AChE system of *T. nivella* (F.) as well as the inhibitory activity of different pesticides on this enzyme. Although our data clearly show that the AChE activity of head homogenates of *T. nivella* (F.) were nearly 25 times greater than bodywall homogenate, the properties of head and bodywall AChE were similar as indicated by the PI_{50} values of the six insecticides. The PI_{50} values of the six insecticides were more or less similar for head and bodywall AChE except that zectran was a stronger inhibitory agent than diazinon in the former. The apparently high activity of AChE in the head may be because of greater concentration of nervous tissue in this region.

Within the dose range tested the effect of the six insecticides was dose dependent. Indeed it has been shown by a number of workers that phosphorylation (METCALF, 1971; CORBETT, 1974) and carbamylation (O'BRIEN, 1960; NISHIOKA *et al.*, 1976) depend upon the inhibitor concentration. Dose dependence also indicates that the concentrations of pesticide were sufficiently in excess of the enzyme, thus making it a pseudo—first order reaction (HART & O'BRIEN, 1973; NISHIOKA *et al.*, 1976).

The progressive increase of AChE inhibition with increase in exposure time of OP compounds indicate that these compounds either combine with AChE very slowly or else are converted into an active form before becoming active AChE inhibitors (FUKUTO *et al*, 1956). MATSUMURA (1976) is of the view that the affinity of P atom to combine at the esteratic site of AChE molecule depends on the electrophilicity of the P atom; OP compounds having P = S group are ineffective as

AChE blocking agent in their native form probably because these are relatively less electrophilic. Contrary to this the P=O metabolites have a more reactive P group with higher electrophilicity.

BOWMAN & CASIDA (1958), O'BRIEN (1960) and YANG *et al.*, (1971) have reported that phorate, malathion and diazinon having P=S group in their molecule first undergo conversion to form corresponding oxygen analogues (P=O) with the help of microsomal mixed function oxidases in order to become active. Similarly, OONNITHAN & CASIDA (1968) have shown that both zectran and carbaryl are oxidised to more active forms by mixed function oxidases. The increase in anti-AChE activity of the pesticides with increase in exposure time in the case of *T. nivella* (F.) may be due to a time related activation of these pesticides. It has been reported by FUKUTO *et al.*, (1956) that enzymatic degradation of the active forms of OP compounds is a slower process as compared to their biotransformation to active forms resulting in a gradual rise in concentration of the latter. DAVIES *et al.* (1970) also showed that the inhibition tends towards a maximum slope as the active inhibitor concentration increased with time in case of fly head AChE. While conversion from P=S to P=O may explain increase in inhibitory potency of the other OP compounds it does not explain the time-dependent increase of AChE inhibition in case of trichlorfon which is P=O compound in its native form, even though it took the longest time (60 min) to cause maximum inhibition. It appears that trichlorfon gets converted into DDVP (METCALF *et al.*, 1959), which is more effective inhibitor of AChE.

Zectran perhaps because of the presence of N-methyl as well as 4-dimethyl group was found to have a greater inhibitory potency as compared to carbaryl which has only N-methyl group (METCALF, 1971). Lower inhibitory activity of carbaryl may also be due to the fact that its structure is dissimilar to AChE and has thus been shown to be only moderately active on isolated enzyme on insects (METCALF, 1971).

An attempt has been made in this study to correlate the AChE inhibitory potency of the six pesticides with the LD_{50} of these pesticides measured earlier by the present authors (PANDEY & AGARWAL, 1980). Although, AChE inhibition seems to be the best explanation for toxic effects produced by OP and Carb. insecticides (CORBETT, 1974; RAINSFORD, 1978), yet we could not find a suitable correlation between the order of toxicity and inhibitory potency of the six compounds. All the six insecticides, though highly toxic, did not show comparable AChE blocking action *'in vitro'*. Similar findings have been given by LORD & POTTER (1954), MENN & HOSKINS (1962), CHADWICK (1963), DAUTERMAN & O'BRIEN (1964) and MORALLO & SHERMAN (1967). This apparent anomaly could be because of the fact that while the *'in vitro'* studies were carried out for 1 h, the LD_{50} values were calculated following 24 h exposure. It is also possible that, these pesticides may be causing death of the larvae not merely by inhibiting AChE but also several other enzymes like chymotrypsin and trypsin (HOLMSTEDT, 1959), enzymes of carbohydrate and protein metabolism (DHAM, 1971; IYENGAR *et al.*, 1972; DATTA & PRAMANIK, 1976; MOCZON, 1976).

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CHEMICAL CONTROL OF BRINJAL SHOOT- AND FRUIT-BORER *LEUCINODES ORBONALIS* GN. WITH NEWER INSECTICIDES

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Five insecticide spray formulations, fenvelerate (sumicidin), carbaryl (sevin), quinalphos (ekalux), chlorpyriphos (dursban) and phosalone (zolon) were tried for the control of the brinjal fruit- and shoot-borer (*Leucinodes orbonalis* Gn.). Taking the per cent incidence of the borer in shoots and fruits and the yields of fruits in both *kharif* and *rabi* seasons, the synthetic pyrethroid fenvelerate at 0.005 and 0.01 per cent and carbaryl at 0.15 per cent proved effective in the control of the fruit- and shoot-borer of brinjal.

(*Key words:* brinjal shoot- and fruit-borer, *Leucinodes orbonalis* Gn., fenvelerate, carbaryl, phosalone, quinalphos and chlorpyriphos)

INTRODUCTION

Among the various pests of brinjal, fruit- and shoot-borer (*Leucinodes orbonalis* GN.) causes severe losses to a tune of nearly 40.0 per cent. Several insecticides were recommended by various earlier workers for the control of shoot and fruit borer of brinjal, but they offer only partial control of the pest. LEELA DAVID (1963, 1964, 1966), GAIKWAD (1969), JOSHI & SHARMA (1973), LAKSHMINARAYANA (1974), NAIR & NAIR (1976), and CHANDURWAR (1979) recommended carbaryl spraying for the control of brinjal shoot and fruit borer. LAKSHMINARAYANA (1974), AYYANA *et al.* (1976) and CHANDURWAR (1979), suggested quinalphos for the control of the pest. The insecticide phosalone was suggested by UTHAMASWAMY *et al.* (1976) and SHAH (1979).

MATERIALS AND METHODS

Field experiments were conducted at commercial farm, College of Agriculture, Rajendranagar during *kharif* and *rabi* seasons of 1978-79 for the control of fruit- and shoot-borer with a highly susceptible *local* variety using 12 treatments in randomised block design with four replications. Plot size was kept 4.10 × 2.5 meters and a spacing of 50×50 cm was adopted in all the plots. The crop was transplanted on 21-9-1978 in *kharif* and 17-3-1979 in *rabi*.

Four insecticidal applications with phosalone at 0.025 and 0.05 per cent, quinalphos 0.025 and 0.05 per cent, chlorpyriphos 0.025 and 0.05 per cent, carbaryl 0.1 and 0.15 per cent, fenvelerate 0.005 and 0.01 per cent. Water spray control and untreated control have been made in *kharif*, 1978 at the ages of 30, 45, 60 and 75 days after planting in both *kharif* and *rabi* 1978. The sprayings extended from 21-10-78 to 2-12-78 in *kharif* and from 16-4-79 to 28-5-79 in *rabi*, 1978. The insecticides were applied as foliar sprays with a hand compression sprayer using 750 ml of spray fluid per plot uniformly in all the applications.

Weekly assessments of the shoot borer infestation were done by counting the total number of healthy and affected shoots in each plot.

Similarly the fruit borer incidence was assessed by recording healthy and affected fruits in each treatment at each weekly picking along with yields both in *kharif* and *rabi* seasons. Thus a total number of three counts for the damage in shoots and six counts for the damage in fruits were taken in both kharif and rabi seasons. The mean values for incidence of the pest and yields in *kharif* and *rabi* are presented in Table 1 after analysing the data statistically.

RESULTS AND DISCUSSION

The initial incidence in the plots on 21-10-1978 in *kharif* and 16-4-1979 in *rabi* before application of the insecticidal sprays was not significant. In *kharif* season, fenvelerate at 0.005 and 0.01 per cent and carbaryl 0.15 per cent were superior to the rest of the treatments in controlling the pest in shoots and fruits besides giving higher yields in *kharif*. The incidence of the pest ranged from 2.76 to 3.54 per cent in shoots and 9.33 to 10.31 per cent in fruits while the same ranged at 10.89 to 11.95 and 37.45 to 41.28 in the corresponding control plots respectively. In respect of yields of fruit

TABLE 1. Per cent infestation by shoot and fruit borer of brinjal and yield data in *kharif* and *rabi* (1978—'79).

Treatments	1978 *kharif*			1978—'79 *rabi*		
	Mean per cent infestation in shoots	Mean per cent infestation in fruits	Yield (kg/ha)	Mean per cent infestation in shoots	Mean per cent infestation in fruits	Yield (kg/ha)
1. Phosalone 0.05% (T_1)	(12.52) 4.75	(36.03) 34.69	3,200	(13.05) 5.15	(33.52) 30.55	2,434
2. Phosalone 0.025% (T_2)	(11.63) 4.25	(33.58) 30.67	2,595	(11.68) 4.17	(32.39) 28.76	2,324
3. Quinalphos 0.05% (T_3)	(10.47) 3.38	(27.69) 21.63	5,746	(11.54) 4.03	(25.84) 19.09	4,505
4. Quinalphos 0.025%	(11.24) 3.80	(31.88) 27.92	4,000	(13.10) 5.25	(29.33) 24.02	3,380
5. Chlorpyriphos 0.05%	(10.63) 3.54	(30.64) 40.74	2,926	(12.66) 4.81	(36.03) 34.62	2,451
6. Chlorpyriphos 0.025%	(14.18) 6.02	(37.52) 37.14	2,000	(12.66) 4.89	(35.43) 33.61	1,898
7. Carbaryl 0.1%	(11.24) 3.83	(28.25) 22.43	5,512	(11.68) 4.17	(26.35) 19.71	4,595
8. Carbaryl 0.15%	(9.81) 2.91	(18.53) 10.15	4,234	(10.14) 3.10	(19.19) 10.82	3,566
9. Fenvelerate 0.01%	(10.76) 3.52	(17.78) 9.33	4,868	(10.94) 3.60	(14.65) 6.40	3,978
10. Fenvelerate 0.005%	(9.46) 2.76	(18.72) 10.31	4,058	(8.72) 2.39	(13.94) 5.87	3,507
11. Control (water spray)	(20.18) 11.95	(39.93) 41.28	2,634	(19.00) 10.69	(41.03) 43.12	2,180
12. Untreated control	(19.19) 10.89	(37.70) 37.45	2,126	(16.85) 8.47	(40.40) 42.00	1,788
C.D. (0.05)	3.56	6.07	1,717	2.96	4.36	1435.93

Note: Figures in paranthesis are transferred angular values.

the three treatments referred to above and quinalphos 0.05 per cent and carbaryl 0.1 per cent gave higher yields ranging from 4058 to 5746 kg/ha which were on par with each other while the other treatments including control recorded only 2000 to 3200 kg/ha which were also on par with each other in *kharif*. In *rabi* crop, fenvelerate 0.005 and carbaryl 0.15 were effective in controlling the pest in shoots and fruits recording from 5.87 to 6.40 per cent while the same was above 40.4 besides giving higher yields. The insecticides carbaryl, fenvelerate and quinalphos at both the concentrations gave significantly higher yields ranging from 3380 to 4595 kg/ha while the same ranged from 1788 to 2451 kg/ha in the rest of treatments. When the incidence of the shoot- and fruit-borer and yields are considered together for both *kharif* and *rabi* seasons, fenvelerate in both the concentrations proved effective. Carbaryl 0.15 per cent was also equally effective and on par with each other in most of the cases agreeing with LEELA DAVID (1963, 1964, 1966), GAIKWAD (1969) JOSHI & SHARMA (1973) and LAKSHMINARAYANA (1974). However the performance of phosalone was at variance with UTHAMASWAMY *et al.* (1976) and SHAH (1979).

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FATE OF ORALLY APPLIED DDT IN DESERT LOCUST *SCHISTOCERCA GREGARIA* FORSK. (ORTHOPTERA : ACRIDIDAE)

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DDT fed to adult desert locust, *Schistocerca gregaria* F. was excreted at a very fast rate (81.66 per cent in 24 hr) and only 14.06 per cent was absorbed. The absorbed DDT was found completely metabolised to DDE in different tissues with maximum concentration in cuticle (48.98 per cent). Head contained significant amount of DDE (16.41 per cent).
(Key words: DDT, feeding, desert locust metabolism)

INTRODUCTION

The natural tolerance of desert locust, *Schistocerca gregaria* F. to DDT has been known since long (MANSINGH, 1957). Electrophysiological studies revealed that the nervous system of this insect is sensitive to DDT (RANGARAO, 1963). In recent years a number of attempts have been made to study various factors contributing to DDT tolerance in *S. gregaria* like its penetration (GULERI & NATH, 1981); distribution (NATH & MEHROTRA, 1980); *in vivo* metabolism (SRIVASTAVA & MANSINGH, 1966; NAQVI *et al.*, 1970), *in vitro* metabolism (NATH & MEHROTRA, 1977) and binding with haemolymph proteins (NATH & MEHROTRA, unpublished data). Since this insect can also tolerate high doses of DDT on feeding, it was therefore, of interest to study the absorption, excretion, distribution and metabolism on oral application of DDT to *S. gregaria*.

MATERIALS AND METHODS

S. gregaria were reared in laboratory at 32±1°C as per method of MEHROTRA & RAO (1966). Adults of both sexes, 20—30 days old and of uniform size were starved for 24 hr before treatment. Oral treatment consisted of three replications with two pairs in each replication. The starved insects were fed with 250 µg DDT per insect through fresh cabbage leaves which were completely eaten within two hours. The insects were then given untreated leaves. Faeces of these insects were collected at different intervals and analysed for DDT. After the DDT was completely excreted, insects were sacrificed, tissues were removed and analysed for DDT and its metabolites. Faeces and tissues were ground with anhydrous sodium sulfate in a pestle and mortar and extracted twice with 25 ml of petroleum ether. The extracts were cleaned by Florisil column chromatography according to LANGLOIS *et al.* (1964) for gas liquid chromatography. For thin layer chromatography, partitioning with petroleum ether and two volumes of acetonitrile followed by Florisil column chromatography, was found adequate. Gas liquid chromatography was done by injecting 4 µl of clean extract in a Toshniwal Model RL 04 OIA equipped with ^{3}H detector and all glass column 180×0.3 cm packed with 5 per cent DC--200 on chrmosorb 60—100 mesh. The conditions were, oven 160°C, injection block 290°C and detector 200°C. In all cases results of GLC were varified by TLC. Thin layer plates were developed with *n*-hexane. Chromogenic reagent was 0.5 per cent silver nitrate followed by ten minute exposure to UV light.

RESULTS AND DISCUSSION

DDT was excreted at a very fast rate i.e, 81.66 per cent was excreted within 24 hr and only 14.06 per cent was absorbed in 96 hr of treatment (Table 1). The observations derive support from earlier results on *Melanoplus differentialis* in which 85 per cent of the applied DDT was excreted within 24 hr, out of which about 10 per cent was found metabolised to DDE (STERNBURG & KEARNS, 1952). Very little metabolism of excreted DDT was however, observed during its passage through the gut in *S. gregaria*.

TABLE 1. Excretion of orally applied p,p'—DDT (250 μg/insect) in adult desert locust, *S. gregaria*.

Time (hr)	Mean DDT* excreted (μg)	Per cent recovery
24	816.67±11.55	81.66
48	31.25± 7.91	3.12
96	11.66± 3.65	1.16
Total DDT excreted	859.58	85.94
Total DDT absorbed	140.42	14.06

*Replication consisted of two pairs of insects.

The DDT absorbed from the gut was found to be evenly distributed, 192 hr after oral application (Table 2). Significantly, all the absorbed DDT got metabolised to DDE and there was no trace of DDT in any tissue as seen in GLC or TLC. This corroborates our earlier findings on the presence of high titres of DDT—dehydrochlorinase in this insect species (NATH & MEHROTRA, 1977). As reported earlier, the maximum DDE (48.98%) was found in the cuticle (NATH & MEHROTRA, 1980). Contrary to our earlier findings, 16.41 per cent DDE in the head was rather high.

The low uptake of DDT on oral application could be due to poor gut absorption because of lipophilic nature of DDT and possibly due to its fast excretion. But accumulation of about four fold more DDE in the head than that reported earlier is intriguing (NATH & MEHROTRA, 1980). The distribution pattern of DDT perhaps depends upon the method of application. The fact that the insect remained unaffected with 22.9 ± 3.75 μg DDE in the head shows that either DDT was metabolised during its passage through the cuticle, which has the highest titres of DDT—dehydrochlorinase in this insect (NATH & MEHROTRA, 1977) or most of it got metabolised in the oral cavity and did not reach the brain. As high as 69 per cent radioactivity was seen in the head of house fly 24 hr of topical application of DDT on gena (LEROUX & MORRISON, 1954). Complete recovery of orally applied DDT during the present investigation indicates that dehydrochlorination is the only metabolic pathway of DDT in this species.

TABLE 2. Distribution on oral appllication of p,p'—DDT (250 μ g/insect) in adult desert locust, *S. gregaria* after 192 hr.

Tissue	Total Mean DDE*	Per cent DDE
Cuticle	68.33±6.06	48.98
Gut	25.00±3.16	17.92
Fat body	23.25±3.67	16.66
Head	22.91±3.75	16.41
Total DDE	139.49	

*Replication consisted of 2 pairs of insects.

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EFFECT OF TWO CHITIN INHIBITORS ON REPRODUCTION OF *TROGODERMA GRANARIUM*

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On application of the compounds A13-29054 and A13-63223 by topical and contact methods to adult *Trogoderma granarium*, a significant fall in oviposition was recorded. Complete sterility was noted at 20 μg/insect with both the compounds following topical application. The compounds were effective to both male and female insects. The topical method was superior to contact method.

(*Key words:* chitin inhibitors, reproduction, *Trogoderma granarium*, diflubenzuron, penfluron)

INTRODUCTION

The newly discovered chitin synthesis inhibitors primarily disrupt the development of the immature stages of a wide variety of insects by interfering with the moulting process. However, they are also known to affect the reproductive capacity of the adult insects and thus, behave as insect sterilants. Reproductive inhibition induced by these compounds has been reported in *Pectinophora gossypiella* SAUNDERS (FLINT & SMITH, 1977), *Ceratitis capitata* WEID (ARAMBOURG *et al.*, 1978), *Anthonomus grandis* BOHEMAN (MOORE & TAFT, 1975; LLOYD *et al.*, 1977), *Musca autumnalis* DEGEER (PICKENS & DEMILO, 1977), *Glossina morsitans morsitans* WESTWOOD (JORDAN *et al*, 1979) and many others. In the present investigation the potentialities of two chitin synthesis inhibitors, A13-29054 (diflubenzuron) and A13-63223 (penfluron), as sterilizing agents have been tested against khapra beetle, *Trogoderma granarium* (EVERTS).

MATERIALS AND METHODS

Khapra beetle was reared in muslin topped one—pound jam jars half filled with wheat flour containing 5% yeast. The jars were kept at a temperature of 35±2°C and 70±5% r. h. The food was oven sterilized to prevent the infestation. 0—24 hours old virgin adults were treated by topical and contact methods.

Topical method: Two μl solution of both the compounds in acetone at 20, 10 and 5 μg insect was applied topically on the ventral side of the adult by a microapplicator. In control the same volume of acetone was applied. *Contact method:* The films of the compounds A13-29054 and A13-63223 were prepared by spreading 1 ml of 1% solution of each compound in acetone in a petridish (5 cm in diameter). The solvent was allowed to evaporate by rotating the petridish. A thin coating of liquid paraffin prevented the insects from coming out of the petridish. The adults were allowed to crawl on the treated surface for different desired periods. A control with acetone was also run.

The treated adults were kept on wheat flour in a plastic vial at 35±1 C and 70±5% r. h. and the effect on fecundity and fertility were studied. The effect on the following combinations were also studied: treated male × treated female; treated female × untreated male; treated male × untreated female.

RESULTS AND DISCUSSION

On administering the compounds A13-29054 and A13-63223 by topical (Table 1) and contact (Table 2) methods, a fall in oviposition of treated females was observed and the mean number of eggs/female was significantly reduced as compared to the control. It is also evident from Tables 1 and 2 that the eggs laid per female were considerably reduced following topical application of the compounds as compared to contact application. The fecundity of the adults treated with acetone was also reduced (34.0 eggs/female) than that of females without any treatment (46.7 eggs/female). It was suggested that on topical application the fecundity of the adults was reduced due to the combined effect of the compound and solvent whereas on contact application only the compound affects as the solvent was made to evaporate. FLINT *et al.* (1978) also reported that exposure of adult *P. gossypiella* at the dose level of 18 mg/cm² of the compounds A13-63223 (penfluron) and A13-63220 resulted in reduced oviposition. According to ARAMBOURG *et al.* (1977) the mean number of eggs laid by female *C. capitata* fed

TABLE 1. Fecundity and fertility of the adults of *T. granarium* treated topically with A13—29054 and A13—63223 at different dose levels.*

Dose µg/insect	Sex treated	A13—29054			A13—63223		
		Total no. of eggs	Mean no. of eggs/female	per cent egg hatching	Total no. eggs.	Mean no. of eggs/female	per cet egg hatching
20	Both	91	18.2±2.78 P<0.02	0	83	16.6±5.35 P<0.05	0
	Female	113	22.6±3.78 P<0.05	15.9	99	19.8±4.80 P<0.05	12.1
	Male	110	22.0±3.69 P<0.05	9.1	97	19.4±4.34 P<0.05	7.2
10	Both	107	21.4±3.58 P<0.05	6.5	88	17.6±4.54 P<0.05	4.5
	Female	115	23.0±3.35 P<0.05	19.1	103	20.6±3.96 P<0.05	15.5
	Male	114	22.8±3.40 P<0.05	17.5	100	20.0±4.53 P<0.05	12.0
5	Both	111	22.2±3.59 P<0.05	9.9	98	19.6±4.05 P<0.05	8.1
	Female	123	24.2±2.84 P<0.05	24.3	107	21.4±4.00 P<0.05	15.8
	Male	118	23.6±3.00 P<0.05	21.1	102	20.4±3.80 P<0.05	14.7
Control (Acetone treated)		170	34.0±3.03	91.1	—	—	—
Control (No treatment)		237	47.4±4.01	98.3	—	—	—

* No of pairs crossed = 5

TABLE 2. Fecundity and fertility of the adults of *T. granarium* treated by contact method with A13—29054 and A13—63223.

Treatment period (Hrs)	Sex treated	No. of pairs	A13—29054			A13—63223		
			Total no. of eggs.	Mean no. of eggs/female	Per cent egg hatching	Total no. of eggs.	Mean no. of eggs/female	Per cent egg hatching
	Both	10	264	26.4±4.8 P<0.001	90.9	00	30.0±3.5 P<0.001	84.3
4	Female	5	170	34.0±3.1 P<0.001	94.1	171	34.2±6.6 P<0.05	91.3
	Male	5	163	32.6±0.9 P<0.001	92.0	161	32.2±4.9 P<0.01	90.5
	Both	10	323	32.3±1.4 P<0.001	92.8	333	33.3±4.9 P<0.01	86.5
2	Female	5	179	35.8±3.4 P<0.01	95.3	184	36.8±5.4 P<0. 5	89.6
	Male	5	166	33.2±2.1 P<0.001	95.4	181	36.2±4.7 P<0.02	92.8
	Both	10	361	36.1±0.7 P<0.001	92.0	371	37.1±4.0 P<0.01	87.0
1	Female	5	199	39.8±3.7 P<0.05	94.1	207	41.4±8.0 NS	94.2
	Male	5	178	37.6±5.4 P<0.05	94.9	191	38.2±6.8 NS	91.7
	Control	10	513	51.3±2.3	97.4	—	—	—

with dimilin treated diet was considerably reduced. On the contrary, several other authors reported that these compounds had no effect on the fecundity of the insects (JORDAN *et al.*, 1979; PICKENS & DEMILO, 1977; REDFERN *et al.*, in press).

Although no marked effect on egg hatching was recorded after contact application of the compounds, it was considerably reduced following topical application. The effect on egg hatching was dose-dependent. Hundred per cent inhibition of egg hatching was recorded at 20 μg/insect of both the compounds (Table 1). It was suggested that considerable reduction in egg hatching following topical application may be due to better penetration of the compound by this method.

These compounds were effective to both male and female insects. The reproductive inhibition was slightly more when treated males were mated with untreated females than when treated females were mated with untreated males. REDFERN *et al.* (in press) also reported that the compounds diflubenzuron, penfluron and A13-63220 were more effective to male than female milkweed bug in reducing hatching of the eggs. They suggested that sterility in male was caused by their inability to transfer sperm, though the mating drive and attempt for copulation appeared unaffected.

Of the two compounds tested, A13-63223 was more effective than A13-29054 in reducing reproductive potential of *T. granarium*. OLIVER *et al.* (1977) also reported that A13-63223 was more effective

than diflubenzuron as boll weevil sterilant. Further, the topical method has been found superior to contact application of the compounds in suppressing the reproductive potential of *T. granarium*.

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STUDIES ON THE FOOD AND FEEDING BIOLOGY OF MAY FLY NYMPHS (INSECTA : EPHEMEROPTERA) AND THEIR ROLE IN AQUATIC ECOSYSTEM

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The gut content analysis of ephemerid nymphs revealed that they were mainly subsisting on algal diet belonging to Chlorophyta, Bacillariophyta and Myxophyta. However, a variety of other food products such as detritus and flagellates also constitute part of their diet. In summer seasons Bacillariophyta were found in maximum percentage (47.8%) while in other seasons Chlorophyta dominated (370.%, 61.0%, and 54.0%). Detritus was recorded in maximum percentage in monsoon season (12.0%). Myxophyta formed only 10.3 per cent of their food items in monsoon season. These nymphs have preference for Chlorophyta and detrital food items, showing their selective nature of feeding, as their concentration in the environmental water was found lower than in the gut.

(Key words: aquatic ecosystem, mayfly nymphs, Chlorophyta, Bacillariophyta, Myxophyta, detritus, energy-flow, biological filters, pollution, prevention)

INTRODUCTION

In recent years, ecologists have shown increasing interest in determining the trophic relations of invertebrates as a necessary prerequisite in studying energy-flow and community dynamics (CUMMINS, 1973). The understanding of dynamic process oriented mechanisms such as food resource partitioning and nutrient spiraling require supportive knowledge regarding food selection, diel and seasonal periodicity, and developmental changes in feeding pattern. Therefore, it is a major problem to identify what food is being eaten by a particular species in its habitat because it is one of the most direct inter-links between the trophic components in an ecosystem. It seems that most species of insects do not feed whatsoever is available in their habitat but actively select their specific food species or food substances. Usually several different kinds of food are eaten during periods of active body growth and reproductive phase (GRODZINSKI *et al.*, 1975).

Literature pertaining to the food and feeding habits of aquatic insects in freshwater bodies is rather scanty, except a few scattered references (WINTERBOURN, 1974; FULLER & STEWART, 1976; SHAPAS & HILSENHOE, 1976; HASAN, 1976). In the present investigation the food and feeding habits of the nymphal stages of Ephemeroptera have been studied in a freshwater pond of Bhagalpur (86°57′13″ EL and 25°14′21″ NL). It is a rectangular pond with a maximum depth of 2.3 m at the centre. The total littoral area of the pond is composed of seasonal macrophytic growth like *Eichhornia crassipes; Potamogeton crispus* and *Hydrilla verticillata.* The

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source of the water of the pond is rain in the catchment area.

MATERIALS AND METHODS

The nymphal stages of Ephemeroptera were collected using an insect collecting net made of nylon cloth (mesh size 40–80/cm²) in different seasons viz., summer, monsoon, winter and spring during the period June 1978 to May 1979. The nymphs were sorted out, killed in 70 per cent alcohol and transferred to 3 per cent formalin for preservation.

The specimens were dissected out under water or 3 per cent formalin. The intact foregut was removed and dissected further on a glass silde. The techniques as suggested by MECON & CUMMINS (1964) and SHAPAS & HILSENHOFF (1976) were used with some modifications to characterise feeding habits. The algal cells, plant tissues, diatoms and detritus were counted using the method of BROWN (1961). Approximate per cent by volume of food items (algae, diatoms, detritus and other matters) was recorded for each season. No attempt was made to analyse midgut contents, since possible misinterpretation of the source of undeterminable matter might result (i. e., what appears as detrital or undeterminable matter in the midgut could have been resulted from proventricular action or partial tdigestion).

Plankton of overlying water were collected by a plankton net made of bolting silk no. 25 (mesh size 0.03–0.04 mm) and counted by LACKY micro-transact counting method (LACKY, 1938).

RESULTS

The seasonal variations in the percentage composition of major dietary components of nymphs of Ephemeroptera have been summarised in the Table. The gut content analysis of three genera of order Ephemeroptera viz., *Cloeon*, *Baetis* and *Ephemerella* revealed that they are only restricted to vegetarian diet. Of the recorded dietary components Chlorophyta, Bacillariophyta and detritus constitute substantially the major food of Ephemeroptera nymphs.

In summer season Bacillariophyta were found in maximum percentage (47.80%) of all food items and next to it were Chlorophyta (32.2%), In monsoon season Chlorophyta were recorded in maximum percentage (37.0%) and Bacillariophyta were next to it (29.2%). In winter and sp.ing seasons Chlorophyta were also in maximum percentage (61.0% and 54.0%). Detritus were recorded in maximum percentage in monsoon season (12.0%). Myxophyta formed only 10.3 per cent of their food items in monsoon season. Flagellates were recorded in very small quantities during other seasons. A small percentage of unidentified matter was recorded during various seasons.

The percentage composition of Chlorophyta in the environmental water varied from 24.5 to 39.8 percent with minimum and maximum recorded in winter and monsoon seasons. Bacillariophyta have higher percentage composition than other

TABLE 1. Showing the percentage composition of algal groups in the gut of ephemerid nymphs and in the environmental water.

Seasons	Chlorophyta %		Bacillariophyta %		Myxophyta %		Flagellates %		Detritus %		Other unidentified matters %
	gut	water	gut	water	gut	water	gut	water	gut	water	
Summer	32.2	39.8	47.8	50.8	3.5	4.2	5.7	4.1	4.2	1.5	7.0
Monsoon	37.0	31.6	29.2	46.9	10.3	9.1	3.5	10.0	12.0	3.5	8.5
Winter	61.0	24.5	25.0	60.6	2.9	8.4	3.5	5.0	3.5	2.0	4.0
Spring	54.0	28.9	29.5	50.1	1.5	11.8	2.3	7.2	8.0	2.5	5.0

food items of these nymphs and varied from 46.9 to 60 6 per cent with minimum and maximum recorded in monsoon and winter seasons respectively. Myxophy a varied from 4.2 to 11.8 per cent with minimum and maximum values in summer and spring seasons respectively. Flagellates varied from 4.1 to 10 0 per cent with minimum and maximum values in summer and monsoon seasons respectively. Detritus varied from 1.5 to 3.5 per cent and their percentage composition was found lowest than algal community in the environmental water (Table).

The algae present in the gut of the various ephemerid nymphs include members of following families:—

Chlorophyta:

Spirogyra sp.; *Oedogonium* sp., *Cosmarium* sp., *Closterium* sp., *Staurastrum* sp., *Microsterias* sp., *Pediastrum* sp.

Bacillariophyta:

Navicula sp., *Synedra* sp., *Pinnularia* sp., *Cymbella* sp., *Fragillaria* sp., *Diatomella* sp. and *Tabellaria* sp.

Myxophyta:

Anabaena sp.

Flagellata:

Euglena sp., and *Phacas* sp.

Chloroplasts were encountered in moderate amounts in the hind end of the gut of these nymphs, which gave the gut a greenish tinge.

DISCUSSION

The ephemerid nymphs as seen in the present investigation were mainly found to subsist on algal diet, belonging to the Chlorophyta, Bacillariophyta and Myxophyta; however, a variety of other food products such as detritus and flagellates were also recorded from the guts. The detritus and flagellates constitute an insignificant part of the total food of these nymphs. Similar food habits were observed for *Baetis rhodani* by BROWN (1961) and in *Baetis foemina* by MOORE (1977c). According to BROWN (1961) the low amount of algae in the gut of these nymphs could be due to food availability or due to niche specialization (MOORE, 1977c). It is clear from the present Table that the percentage composition of Chlorophyta and Bacillariophyta in the environmental water was moderate on which the nymphs graze. The detritus and other food items were available only in very low percentage in the environment. Thus, the elevated level of algae present in the gut of these nymphs in various seasons of the year reflects the food availability in the niche where the nymph lives.

These nymphs are known to be mostly free-swimming forms moving to and from amongst the aquatic weeds. Naturally, they are more liable to utilize the planktonic algae and the suspended detritus particles present in such a shallow undisturbed pond. During the present study it has been seen that almost all the groups of algae which occurred as plankton were recorded in the guts of these nymphs (Fig.). This is in conformity with the studies carried out by a number of workers (JONES, 1950; DUNN, 1954; POPHAM, 1955; BROWN, 1961; MOORE, 1977a,b,c). According to POPHAM (1955) in artificial and small ponds, these nymphs were found to subsist mainly on phytoplanktonic and periphytonic algae, whereas in running water situations detritus and vascular plant tissues seem to be the major food (BADCOCK, 1949; JONES, 1950; BROWN, 1961). In the present study detritus and vascular plant tissues formed insignificant percentage of the food items of these nymphs. According to MOORE (1977c) the percentage

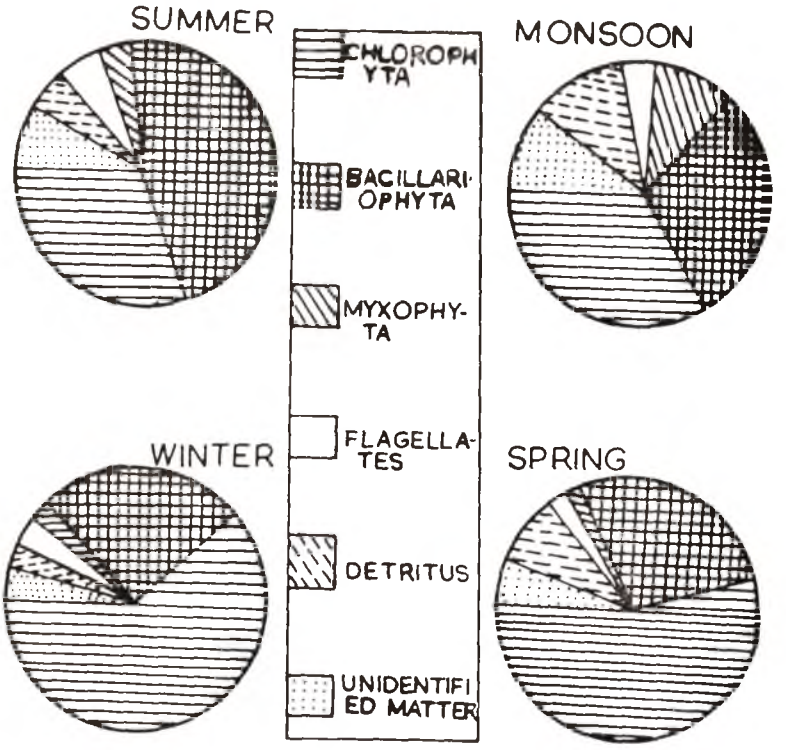

Fig. 1. Gut content of mayfly nymphs.

of algae in the gut was found to be extremely variable between different genera of ephemerid nymphs.

Present Table shows that the percentage composition of Chlorophyta in the environmental water is found lower than the percentage in the gut content. In winter and spring seasons the percentage composition of Chlorophyta in the gut was found 61.0 per cent and 54.0 per cent whereas in environmental water it was 24.5 per cent and 28.9 per cent respectively. The percentage composition of Bacillariophyta in environmental water was found higher than in the gut content in various seasons of the year. Myxophyta and flagellates were also found in maximum percentage in the environmental water than in the guts of these nymphs. The percentage composition of detritus in the environmental water was found to be low in comparison to the gut.

These observations point towards some sort of selective feeding for algal and detrital food items, although it may be due to non-random distribution or variable local concentrations of these food items. Moreover, these nymphs are known to be active free swimming forms, and they are exposed to a wide variety of food types and the variable local concentrations of planktonic groups makes it difficult to comment on selectivity.

Thus, the feeding habits of nymphal stages of Ephemeroptera is complicated by their generalized feeding capabilities. Indeed, food may never become the factor limiting kinds of nymphs present in an aquatic habitat, since within certain broad

limitations, most species can use a variety of food materials for nutrition. This ability to use a range of materials probably encourages exploitations of the aquatic environment to full extent. Further, detritus feeder nymphs have been found to be useful in the prevention of pollution of the inland waters as they feed on decaying organic materials. Thus, they act as biological filters in the purification of natural waters.

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POLYTOXUS SP. (HETEROPTERA), THE SECOND REDUVIID GENUS WITH *XO* MALES

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Males of *Polytoxus* sp. contained in their testes *2n* = 23 *(22 + XO)* and *n* = 12 *(11 + XO)* chromosomes which happened to be the second genus, the first being *Ectrychotes*, to have *XO* males among 88 species belonging to 44 genera under 12 subfamilies of Reduviidae so far cytologically investigated. Except these two genera, all other had *XY* or multiple sex chromosomes in males. Unlike *Ectrychotes* the single *X* chromosome of *Polytoxus* sp. showed the postreductional meiosis, characteristic of Heteroptera. The meiotic behaviour of chromosomes and their metrical data have been presented.

(Key words: post-reductional *XO* male reduviid bug, *Polytoxus* sp.)

INTRODUCTION

Some 88 odd species belonging to 44 genera under 12 subfamilies of Reduviidae so far investigated (see UESHIMA, 1979; MANNA & DEB-MALLICK, 1981) could cytologically be characterized by the modal number of 20 autosomes, the sex chromosome constitution in males ranged between *XO* (rare), *XY* and multiple *X*'s and a *Y*, postreductional meiosis, the sex chromosome number if more than one, they characteristically occupied the central position surrounded by autosomes in the hollow spindle at metaphase II forming a pseudo-bivalent or pseudo-multivalent structure which depended on the sex chromosome constitution and at metaphase I the position of the univalent sex chromosomes was variable among the evenly distributed autosomal bivalents (MANNA, 1956, 1962). Some amount of variation in the cytological characterizations of the family Reduviidae made above was seen in the genus *Ectrychotes* (MANNA, 1951; MANNA & DEB-MALLICK, 1980) and in *Polytoxus* sp. under present report, both had males with *XO* sex chromosome. The occurrence of *XO* males with prereductional meiosis in *Ectrychotes* (MANNA, 1951; MANNA & DEB-MALLICK, 1980) and postreductional meiosis in *Polytoxus* would support the remark made by MANNA (1956, 1962) that the family Reduviidae has been heterogeneously constituted and that would find further support by the work of UESHIMA who arrived at the same conclusion (UESHIMA, 1966, 1979). Since *Polytoxus* sp. is the only species studied in the subfamily Saicinae, if the postreductional meiosis in *XO* males was characteristic either of the genus or the subfamily was not known. Further, if this was found to be characteristic of both, the prereductional *XO* mechanism in *Ectrychotes* would then characterise the genus and the subfamily Ectrychodiinae.

[1] Grateful acknowledgements are made to the Zoological Survey of India, for the identification of the bug and the UGC for financial assistance.

MATERIAL AND METHODS

Six adult males of *Polytoxus* sp. (Saicinae, Reduviidae) were captured at night near the electric lamp at Kalyani, West Bengal and their testes were fixed in acetic-alcohol mixture (1 : 3). Each testis was squashed separately and stained in iron-alum haematoxylin according to the method described earlier (MANNA & DEB-MALLICK, 1980). The metrical data of chromosomes of metaphase I were taken following the method described by MANNA (1951) and the classification of size based on th relative percentage volumes was made according to the arbitrary norm followed by us here and elsewhere as very large (25.1% and above = VL), large (20.1% — 25% = L_1, L_2), medium (15.1%—20% = M_1, M_2), submedium (10.1% — 15% = SM_1, SM_2), small (5.1% — 10% = S_1 S_2) and very small (1% — 5% = VS_1, VS_2).

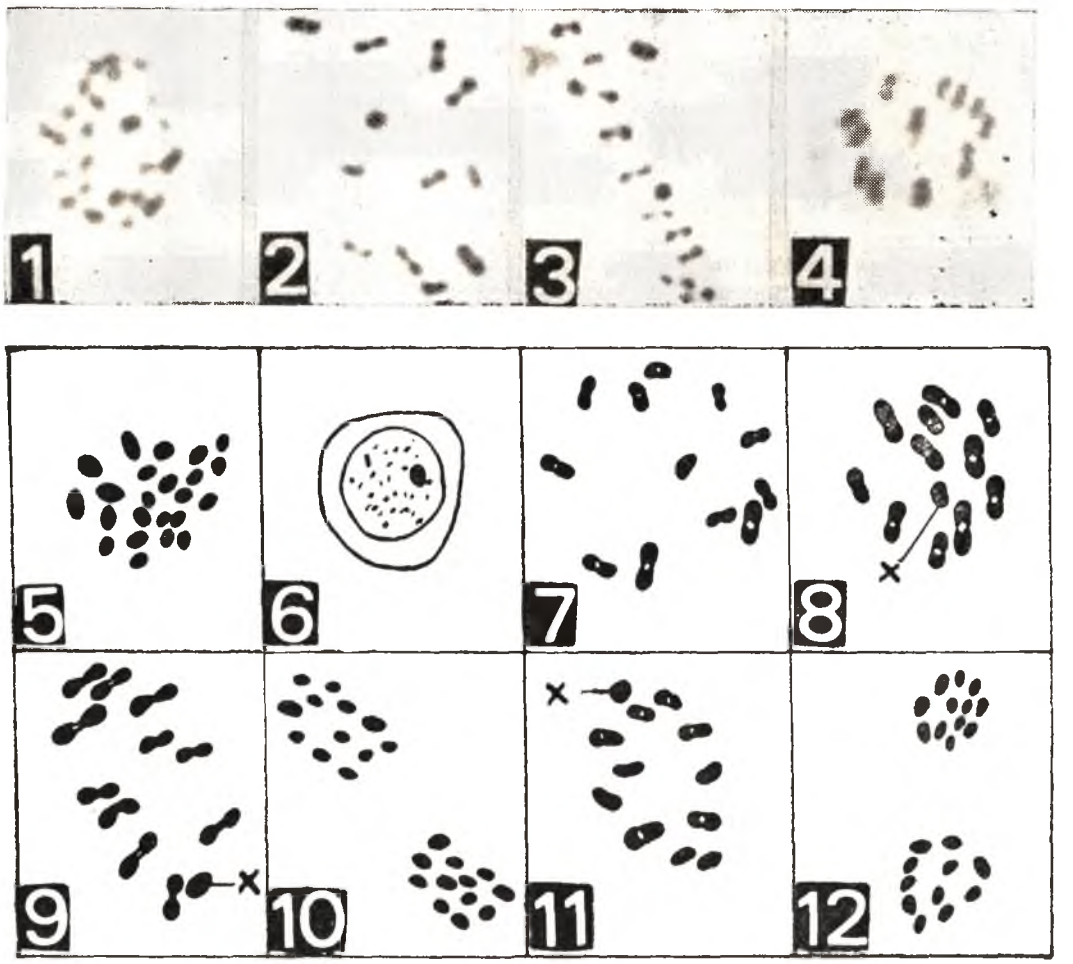
OBSERVATIONS

The spermatogonial metaphase complements (Figs. 1, 5) contained 23 small chromosomes which could arbitrarily put into two groups as 4 medium and submedium and 19 small but the difference was not so obvious. From the odd number and the behaviour of chromosomes during spermatogenesis, it was revealed that the species contained only one *X* which belonged to the smaller group. The single *X* could not individually be indentified by size difference among 19 gradually seriated small chromosomes. It also did not show any differential staining behaviour in the spermatogonial metaphase.

During early first spermatocyte prophase, the nucleus generally contained a small positively heteropycnotic element often close to the nuclear membrane which possibly represented the single *X* element (Fig. 6). The number of elements in prophase I could conveniently be determined at the diakinesis stage (Figs. 2, 7) which contained 11 autosomal bivalents, (two appeared little large) and the smallest univalent element, somewhat individually identifiable, was the *X*. At metaphase I, because of the over-condensation the difference between the autosomal bivalents and the univalent *X* was little less appreciable (Figs. 3, 8, 9). The univalent *X* did not occupy any typical position in the spindle among evenly distributed autosomal bivalents but its relatively small size often made its identity clear. Anaphase I was equational for the *X* and reductional for the autosomes for which each daughter half received 11 autosomes plus the *X* (Fig. 10). The arrangement of 11 autosomes at metaphase II was more typical as they formed a ring round the hollow spindle at the equatorial region while the *X* had variable position. It mostly formed an accessory plate (Fig. 11) and sometimes was lying within the autosomal ring (Fig. 4). As characteristic of Heteroptera, anaphase II was reductional for the *X* chromosome, as a result each daughter

TABLE 1. Mean number of graphical squares and relative percentage volume of chromosomes of metaphase I of *Polytoxus* sp.

	Autosomal number											Sex
	1	2	3	4	5	6	7	8	9	10	11	chrom
Mean no. squares	158	120	87	72	70	68	60	58	55	54	52	32
Relative % volume	17.8	13.5	9.7	8.1	7.9	7.6	6.7	6.5	6.1	6.0	5.8	4.2
Type	M_1	SM_1	S_1	S_1	S_1	S_1	S_2	S_2	S_2	S_2	S_2	VS_1



Figs. 1—12: meiotic chromosomes of *Polytoxus* sp. 1—4: Photomicrographs. 5—12. Camera lucida drawings, × Ca. 3,000. 1 & 5: Spermatogonial metaphase with 23 chromosomes; 6: Early prophase I showing positively heteropycnotic *X* chromosomes; 2 & 7: Diakinesis with 12 elements; 3, 8 & 9: Metaphase I with 12 elements; 10: Anaphase I, each pole with 12 chromosomes; 4 & 11: Metaphase II with 12 elements; 12. Anaphase II showing distribution of M and 12 chromosomes.

half received 11 autosomes but one half plus the *X* (Fig. 12).

The metrical data of mean volume and its relative percentage of chromosome of metaphase I in *Polytoxus* sp. (Table 1) yielded 1 medium, 1 submedium, 9 small autosomes and the very small *X* chromosome. The difference between different size-group was not so apparent if considered superficially. Further the *X* chromosome measured smallest of all but it was really close to the size of autosome no 3 or 4 because the latter were in paired condition in metaphase I. The chromosome formula based on the metrical data was $n = 1M_1 + 1SM_1 + 4S_1 + 5S_2 + 1VS_1$.

DISCUSSION

The absence of the *Y* chromosome and the presence of only one *X* in the two, so to say cytologically aberrant genera *Ectrychotes* (MANNA, 1951; MANNA & DEB-MALLICK, 1980) and *Polytoxus* sp. reported here would suggest their origin at different time scale during their evolution. In connection with the origin of the *XO*: *XX* sex chromosome mechanism in two species of *Ectrychotes*, it was suggested (MANNA & DEB-MALLICK, 1980) that the loss of *Y* took place before the post-reductional meiotic mechanism was established in Heteroptera. Thus, in *Ectrychotes* the prereductional *XO* mechanism in males was the case of retention of the original time of division mechanism in the sex chromosome (prereductional) in which case the *Y* was eliminated earlier. Alternatively the origin of the prereductional meiosis in *Ectrychotes* could be the case of reversion of postreductional meiosis as WHITE (1973) suggested to explain other cases of prereductional meiosis in *Pachylis gigas* (SCHRADER, 1932), *Archimerus calcarator* (WILSON, 1905, 1909) etc. In that case the evolution of *XO* mechanism in male *Ectrychotes* would involve two steps, first the reversion to prereductional meiosis and then the elimination of the *Y* or *vice-versa*. Anyhow, the origin of the *XO* mechanism in male *Polytoxus* sp. would not be difficult to imagine if we considered simply the loss of the *Y* of the original *XY* sex chromosomes with post-reductional mechanism, the characteristic of Heteroptera. This would explain the retention of the postreductional meiosis after changing from *XY* to *XO* in male. The cytological study of *Polytoxus* showed not only the change of sex chromosome constitution but there was also change in autosomes as compared to the modal number of 20 found in Reduviidae. This change could be surmised due to fragmentation of autosomes but as no other congeneric species was studied, we could not suggest more precisely.

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EFFECT OF SPACE ON FOOD UTILIZATION AND MORPHOLOGICAL FEATURES OF THE BUTTERFLY *POLYDORUS ARISTOLOCHIAE* (LEPIDOPTERA : PAPILIONIDAE)

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Reduction in the volume of the container produced a number of negative effects on the butterfly *Polydorus aristolochiae* such as heavy mortality, extension of larval (23 to 27 days) and pupal periods (13 to 16 days) and decrease in the final body weight of the larva (884 to 733 mg live weight). Test individuals reared at 30, 60, 180 380, 580, 1000 and 2500 cc consumed 1184, 1265, 1261, 1315, 1304, 1328 and 1464 mg dry weight of *Arisrolochia bractiata* leaves respectively. The total weights of exuviae were 16.3, 16.9, 16,7, 17·6, 16.8, 17.9 and 18.0 mg for the test larvae reared at different volumes. An increase in the volume of the container caused an increase in the length and breadth of forewings and hind wings and also in the antenna length and body length.

INTRODUCTION

Information on the influence of ecological factors on nhe bioenergetics of insects is plenty in the literature (e.g. MADHAVAN & PANDIAN, 1975; MATHAVAN & BHASKARAN, 1975; HANIFFA & ELAYAPPAN, 1981; HANIFFA & PERIASAMY (1981). But to our surprise no paper is available on the influence of space on the energy budget of lepidopterans and the present paper is the first of its kind. Previous authors (MATHAVAN & NAMBIRAJAN, 1976; HANIFFA & ELAYAPPAN, 1981) who have reported the effects of crowding on energy budget of insects just mentioned that the decrease in energy budget was due to the availability of space; in their experiments on crowding they did not give considerable importance to study the effect of space. According to WALDBAUER (1968) the size of the container in which the experiment is carried out may be of importance (see also FEWKES, 1960; HANIFFA, 1980). Hence an extensive investigation was undertaken to study the influence of space on food utilization and morphological features of the butterfly *P. aristolochiae*.

MATERIALS AND METHODS

Eggs of *P. aristolochiae* were collected from the leaves of *A. bractiata* plants and reared in reparate terraria (capacity: 3 l) in the laboratory for a few days upto hatching. About 21 freshly hatched first instar larvae were recruited and divided into 3 series; each series was divided into 7 groups (each group with one individual) and placed in glass jars of different volumes. i. e., 30, 60, 380, 580, 1000 and 2500 cc.

The test individuals were fed with fresh leaves of *A. bractiata*, *ad libitum*, both in the morning and in the evening. Every day a control sample 1g *A. bractiata* was dried at 80°C for 24 hours to determine the water content (HANIFFA & PERIASAMY, 1981). Unconsumed leaves and faecal pellets were collected every day, dried and weighed to estimate the amountof food consumed and assimilated. A control sample of 10

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first instar larvae were dried at 80°C to determine the initial weight of the first instars. The quantity of food assimilated for each instar was estimated by subtracting the weight of faeces from the weight of food consumed and that of conversion by subtracting the initial live weight of the larvae at the commencement of each instar period from the live weight of the succeeding instar. Data obtained for the change in live wight were multiplied by the factor 0.3942 to obtain the corresponding dry weight.[1] The sacrifice method (MAYNARD & LOOSLI, 1962) was used to measure the initial and final dry weight of the test larvae.

The scheme of energy budget followed here is the slightly modified IBP formula (PETRUSEWICZ & MACFADYEN, 1970) represented as C=P+R+F, where C is the food consumption, P the growth (conversion), R the weight loss due to metabolism and F the undigested food including the nitrogenous excretory products (WALDBAUER, 1968). A stock of larvae corresponding to the test individuals was separately maintained. When experimental larvae died, substitute of the same size were recruited from the stock and introduced (see HANIFFA & PERIASAMY, 1981).

RESULTS

Larval and pupal periods

Figure 1 reveals the influence of space on larval and pupal periods of *P. aristolochiae*. An increase in the volume of the container caused a decrease in the larval and pupal periods. The difference in the first and second instar periods was not remarkable, whereas the remaining instars showed a decrease in the instar periods. For instance the third instar period was 5 days at 30 cc and it decreased to 4.5 days in 2500 cc. The corresponding decreases in the fourth and fifth instars were from 5.5 to 4 days and 7.5 to 6 days. The total larval period decreased from 26.5 days at 30 cc to 23 days at 2500 cc. Data obtained for the pupal periods were 16, 15, 13.5, 14, 13 and 12.5 days at different volumes respectively (Fig. 1).

[1] Each instar was weighed soon after moulting and the corresponding exuvia was also dried and weighed.

Overall energy budget

Data obtained for the energy budget of the first instars reared at different volumes did not show much variation. An increase in space caused an increase in the energy budget of the remaining instars. For instance the second instar consumed 27 ± 2.6 mg dry weight/instar at 30 cc and 31.9 ± 5.9 mg/instar at 2500 cc. The corresponding increases for the third, fourth and fifth instars were from 62 ± 2.9 to 81 ± 1.3 mg/instar, 236 ± 1.4 to 288 ± 28.3 mg/instar and 852 ± 2.4 to 996 ± 25.1 mg/instar. Data obtained for assimilation, metabolism and conversion also showed an increase due to the influence of space (Table 1). The amount of food converted increased from 0.7 to 1.7, 4 to 5, 11 to 19, 49 to 61 and 223 to 290 mg/instar with the increase in space. Assimilation efficiency ranged from 61 to 69% for the different instars. Conversion efficiency ranged from 16 to 42%.

Figure 2 shows the overall energy budget of the test larvae at different volumes. Data reported for food consumption during the total larval periods were 1184, 1265, 1261, 1315, 1304, 1328 and 1464 mg at different volumes respectively. The corresponding values for conversion were 525, 557, 566, 563, 567, 555 and 587 mg.

Exuvia collected for the first instar at 30 cc was 0.8 ± 0.07 mg dry weight and it increased to 1 ± 0.07 mg dry weight at 2500 cc. Corresponding increases from 1.5 to 1.7, 2.4 to 3.4, 4.7 to 5.1 and 7.1 to 7.2 mg were reported for the second, third, fourth and fifth instars. The total weights of exuviae were 16.3, 16.9, 16.7, 17.6, 16.8, 17.9 and 18.0 mg at different volumes respectively (Table 2).

Figure 3 shows the live weight of different instars at various volumes. With

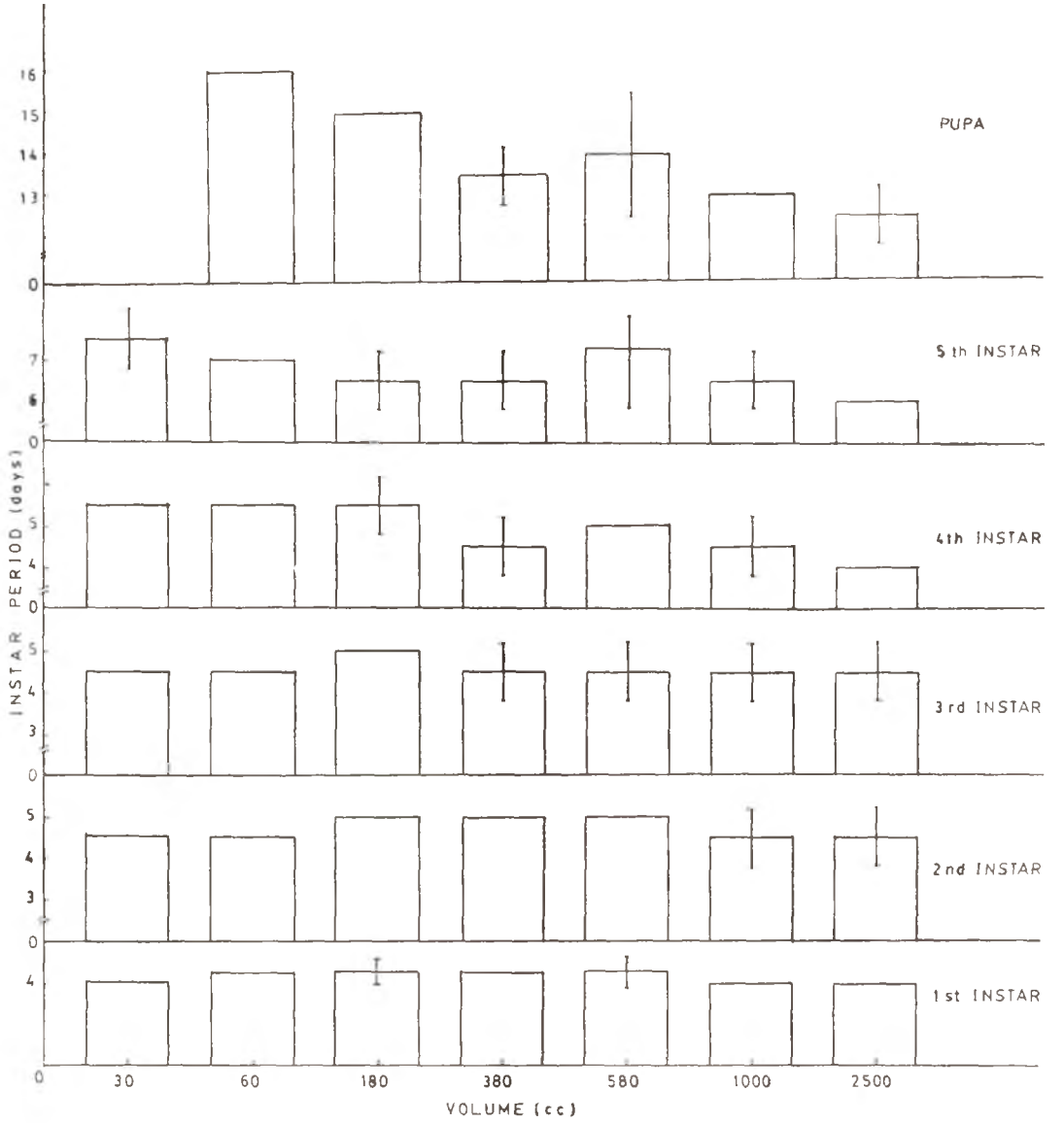

Fig. 1. Influence of volume of the container on larval and pupal periods of *Polydorus aristolochiae.*

the decrease in volume of the container, final body weight attained by the test individuals decreased remarkably. The live weight of the test individuals reared at 2500 cc increased from the mean initial weight of 4.4 ± 0.07 mg to 884 ± 43 mg at the end of the fifth instar and became pupa. Those reared at 30, 60, 180, 380 580 and 1000 cc attained 733, 795, 795 835, 837 and 856 mg and became pupa.

Morphological features

Fig. 4 reveals the length and breadth of fore and hind wings and length of

TABLE 1. Effect of volume of the container on the energy budget of the butterfly *Polydorus aristolochiae* fed with *Aristolochia bractiata* at 30±2°C. Each value represents the average performance of 3 individuals. Values represented as ± indicate the standard deviations.

Volume (cc)	Food consumed	Food assimilated	Food converted	Food metabolized	Absorption efficiency	Conversion efficiency
	(mg dry weight/live instar/day)				(%)	
			1st instar			
30	7± 0.3	5± 0.2	0.7± 0.2	4± 0.1	66± 2	16±4.0
60	7± 0.5	5± 0.3	0.9± 0	4± 0.3	69± 4	1±60
180	8± 0.2	5± 0.2	0.9± 0.1	5± 0.4	67± 0	17±2.1
380	6± 0.9	4± 0.6	0.7± 0.1	4± 0.5	67± 0	17±2.7
580	7± 0.5	5± 0.3	0.8± 0.1	4± 0.2	69± 4	17±2.4
1000	8± 0.4	5± 0.2	0.9± 0.1	4± 0.1	68± 3	17±2.7
2500	7± 0	5± 0	1.0± 0.1	4± 0	69± 0	17±0.6
			2nd instar			
30	27± 2.6	19± 0.9	4.0± 0.1	15± 0.8	69± 4	21±0
60	34± 2.5	23± 1.7	5.0± 0.1	18± 1.6	69± 8	21±0.5
180	37± 1.9	27± 1.2	6.0± 1.2	21± 0	61± 8	21±4.7
380	27± 3.2	17± 2.2	4.0± 0.1	13± 2.1	61± 8	23±0.3
580	27± 1.4	20± 2.3	4.7± 0.3	15± 2.0	69± 8	24±1.4
1000	31± 0	21± 0	5.0± 0.2	16± 0.3	69± 0	24±1.1
2500	31± 5.9	21± 4.0	5.0± 0.3	17± 3.7	69±13	21±1.7
			3rd instar			
30	62± 2.9	43± 1.6	12± 2.3	31± 0.7	69± 3	27±5.4
60	66± 2.9	45± 1.9	12± 2.1	34± 0.2	69± 3	25±4.7
180	81± 0.6	56± 0.4	16± 4.8	40± 0.7	69±13	28±8.6
380	80±15.3	55±10.5	18± 0.2	40±10.3	68±13	26±0.3
580	87± 4.7	60± 2.3	14± 0.8	46± 1.5	69± 8	24±1.4
1000	96± 3.4	66± 2.3	19± 3.1	47± 0.7	69± 3	29±4.7
2500	81± 1.3	50± 0.2	14± 5.8	36± 5.6	62± 3	29±9.7
			4th instar			
30	236± 1.4	162± 0.9	49± 0.9	113± 7.6	69±3	30±0.6
60	240± 5.6	165± 3.3	52±10.3	113± 7.0	69±1	31±6.3
180	242± 7.0	166± 4.9	53± 3.6	113± 1.2	65±2	32±2.2
380	259± 8.9	178± 5.6	53±16.1	116±10.5	69±2	31±0.1
580	264± 2.7	182± 1.9	53± 1.4	129± 0.5	69±1	29±0.8
1000	270± 9.9	165± 6.8	59± 2.1	106± 0.6	61±3	36±1.3
2500	288±28.3	198± 8.6	61± 1.6	137± 7.0	68±4	41±0.8
			5th instar			
30	852± 2.4	585±15.0	223± 5.0	363± 9.7	69± 2	38±0.9
60	918±16.6	630±12.0	243± 5.0	386± 7.0	69±2	39±0.8
180	893± 7.5	614± 5.1	237± 7.0	377± 1.9	69± 1	39± 1.1
380	918±28.0	630± 4.5	256±11.3	397± 6.8	66±1	40±1.8
580	918± 6.5	645±19.3	271±17.1	369± 2.1	69±2	41±2.7
1000	924± 8.1	634± 5.6	252±23.0	383±17.1	69±1	40±3.6
2500	996±25.1	684±17.2	290±17.2	394±27.1	69±2	42±2.5

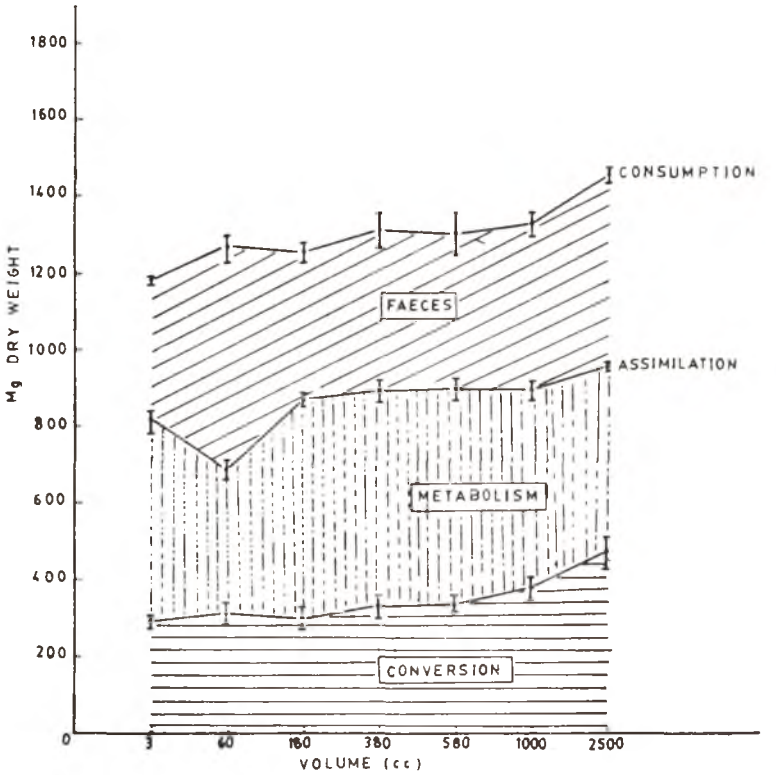

Fig. 2. Overall energy budget of *Polydorus aristolochiae* as a function of space.

TABLE 2 Exuvia weight of different instars of the butterfly *Polydorus aristolochiae*. All values are presented in mg dry weight. Values reported as ± indicate the standard deviation.

Volume (cc)	Instar 1	2	3	4	5	Total weight
30	0.8±0.07	1.6±0.07	2.4±0.07	4.7±0.64	7.1±0.07	16.3±0.92
60	0.8±0.14	1.4±0.06	3.1±0.05	4.7±0.64	7.1±0.07	16.9±0.96
180	0.9±0.07	1.6±0.49	2.4±0.04	5.0±0.07	7.0±0.42	16.9±1.19
380	0.9±0.07	1.6±0.14	2.9±0.35	4.6±0.50	7.6±0.28	17.6±1.34
580	0.8±0.08	1.5±0.14	2.8±0.07	4.5±0.21	7.3±0.56	16.8±1.06
1000	1.0±0.09	1.9±0.07	3.3±0.14	4.9±0.08	6.9±0.28	17.9±0.66
2500	0.8±0.07	1.7±0.05	3.4±0.07	5.4±0.21	7.2±0.50	18.5±0.90

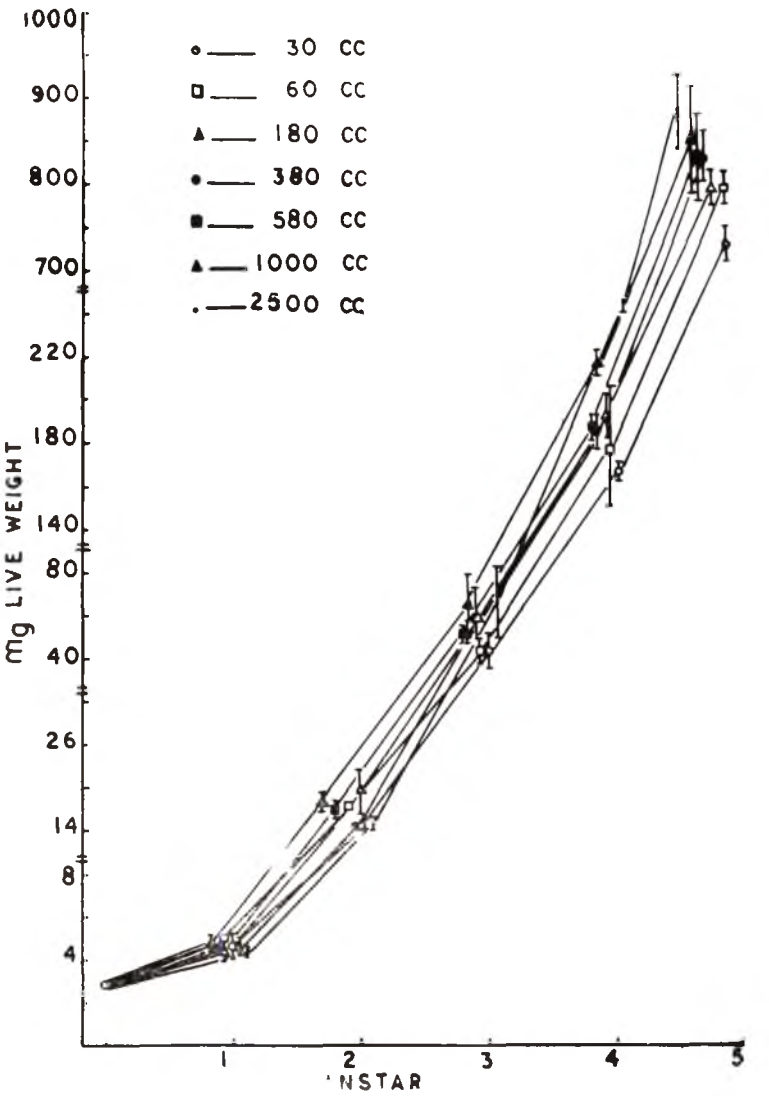

Fig. 3. Growth of *Polydorus aristolochioe* (in live weight) as a function of space.

antenna and body as a function of space. The length and breadth of forewing were 3.7 and 1.7 cm for *P. aristolochiae* reared at 60 cc. The same were 4.3 and 2.1 cm for the test individuals reared at 2500 cc (Fig. 4a). The hind wing length at 30 cc was 3.2 cm and it increased to 4 cm at 2500 cc. The corresponding increase for the breadth was from 1.7 to 1.9 cm (Fig. 4b). An increase in space also caused an increase in antenna length and body length. For instance antenna length increased from 1.4 to 1.8 cm and body length increased from 2.4 to 2.8 cm with the increase in volume of the container from 60 to 2500 cc (Figs. 4c and d).

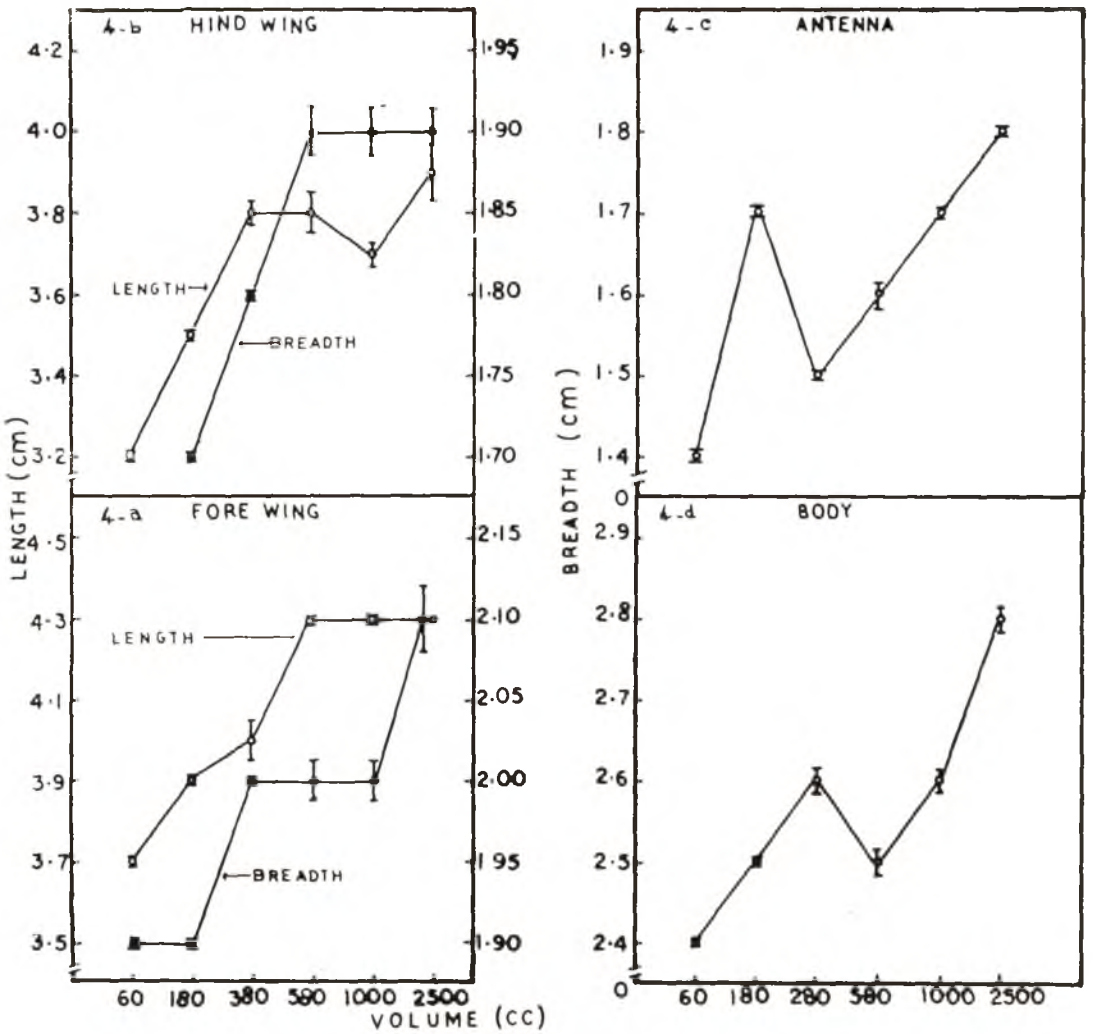

Fig. 4a. Effect of of space on the length and breadth of fore wings of *Polydorus aristolochiae*; 4b. Effect of space on the length aud breadth of hind wings of *Polydorus aristolochiae*; 4c. Effect of space on the length of antenna of *Polydorus aristolochiae*; 4d. Effect of spaee on the body length of *Polydorus aristolochiae*.

DISCUSSION

The present work has a few limitations. For instance the effect of space on the weight or the morphological features of the pupae was not studied, since handling of pupa affects hatching and the aim of the present investigation is to study the morphological features of the adult as a function of space. According to HANIFFA (1980) space is an important ecological factor required by the organism in its interaction with the environment. An increase in food utilization was reported in the snail *Pila globosa* with the increase in volume of the water supplied (HANIFFA, 1980). According to REDDY & PANDIAN

(1973) an increase in volume of water caused an increase in the consumption of mosquito larvae by the fish *Gambusia affinis*. Similar type of work on insects is totally wanting and the present paper is the first of its kind. According to FEWKES (1960) and WALDBAUER (1968) the size of the container is of considerable importance regarding food utilization. In the present investigation an increase in space caused an increase in the length of fore wings, hind wings, antenna and body and breadth of fore and hind wings. MATHAVAN & NAMBIRAJAN (1976) and HANIFFA & ELAYAPPAN, (1981) reported a decrease in fore wing length as a function of crowding. Previous authors also reported that crowding acts through the availability of space and affects feeding (e. g., HANIFFA & ELAYAPPAN, 1981). According to MATHAVAN & NAMBIRAJAN (1976) crowding is known to reduce feeding extent, larval duration and reduce wing length mainly through the "conditioning effort" of larval density and the accumulated excreta in the terraria. But in the present experiment density factor is kept constant while the space is altered and the excreta is removed daily. Therefore it is clear that space plays a significant role in food utilization instead of excreta. HANIFFA (1980) reported an increase in the metabolic activity of the snail *P. globosa* with the increase in volume of the water. Hence it is possible to suggest that the increase in metabolic demand due to the increase in space may induce the larva to seek and obtain more food energy to compensate the higher metabolic loss. Further investigation of this aspect is in progress.

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DISTRIBUTION OF CONSTITUTIVE HETEROCHROMATIN IN *PERIPLANETA AMERICANA* (LINNAEUS) (DICTYOPTERA : BLATTINAE)

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Karyotype of *Periplaneta americana* is described. Karyotype consists of 10 pairs of metacentrics and 6 pairs of subtelocentric autosomes and a large metacentric *X*-chromosome. Distribution of heterochromatin is described. Reorganised karyotype of this species is considered to be the standard.

(Key words: Periplaneta americana, chromosomes, heterochromatin, standard karyotype)

INTRODUCTION

Chromosome biology of *Periplaneta americana* has been the subject of controversy right from the beginning of this century (MORSE, 1909). Later more information has accrued on the chromosome morphology, meiotic sequences and on the occurrence of heterozygosity (SUOMALAINEN, 1946; SHARMA *et al.*, 1956; JOHN & LEWIS, 1957, 1958; SHARMA *et al.*, 1959; DAS GUPTA, 1959; JOHN & LEWIS, 1960; RAJASEKARASETTY & RAMANAMURTHY, 1963, 1964; COCHRAN & ROSS, 1967; KUMARASWAMY & RAJASEKARASETTY, 1976; MAJUMDAR *et al.*, 1976). Further the studies on the nature and distribution of heterochromatin for the chromosomes of Cockroaches has not been made. In an attempt to understand the distribution of heterochromatin, karyological studies on *Periplaneta americana* was undertaken. A comparison of the present karyotype with that given by KUMARASWAMY & RAJASEKARASETTY (1976) revealed certain important differences. Therefore, in the present communication the revised karyotypic details and the distribution of the heterochromatin are described.

MATERIALS AND METHODS

Male and female specimens (10 males and 5 females) were collected from the University campus, Manasa Gangotri, Mysore. The chromomosomal preparations were made from the testis and hepatic caeca by colchicine-hypotonic-air dry-Giemsa staining technique. Localisation of constitutive heterochromatin was made according to Summer's technique (1972) with minor modifications. The karyotypes were made for both sexes and idiogram constructed and is presented (Fig. C).

RESULTS AND DISCUSSION

The diploid number is 33 in males and 34 in females. The karyotypes (Figs. A and B) consist of 10 pairs of metacentrics and 6 pairs of subtelocentric autosomes. The *X* is the largest in the chromosome complement and is metacentric. In male meiosis 16 bivalents and an univalent *X*-chromosome are noticed (Fig. E).

In the *C*-banded karyotype (Fig. D) the *C*-positive regions can be clearly seen

at the centromeric regions of all the chromosomes, but in the last 4 pairs of subtelocentrics the *C*-bands appear to be large. Translocation heterozygosity is not uncommon in this species. In the *C*-banded diakinesis (Fig. F), the *C*-positive regions are seen in all the bivalents and also in the ring chromosome.

According to KUMARASWAMY & RAJASEKARASETTY (1976) the last 6 pairs of autosomes and the *X*-chromosome are submetacentrics. But, the present critical study supplemented by *C*-banding data clearly indicates that the last 6 pairs of autosomes are subtelocentrics and the *X*-chromosome is certainly metacentric in nature and thus, confirms the earlier reports of DAS GUPTA (1959) and MAJUMDAR *et al.* (1976).

Hence, with the above information in hand, it is reasonable to accept the present reorganised karyotype of *Periplaneta americana* as the standard.

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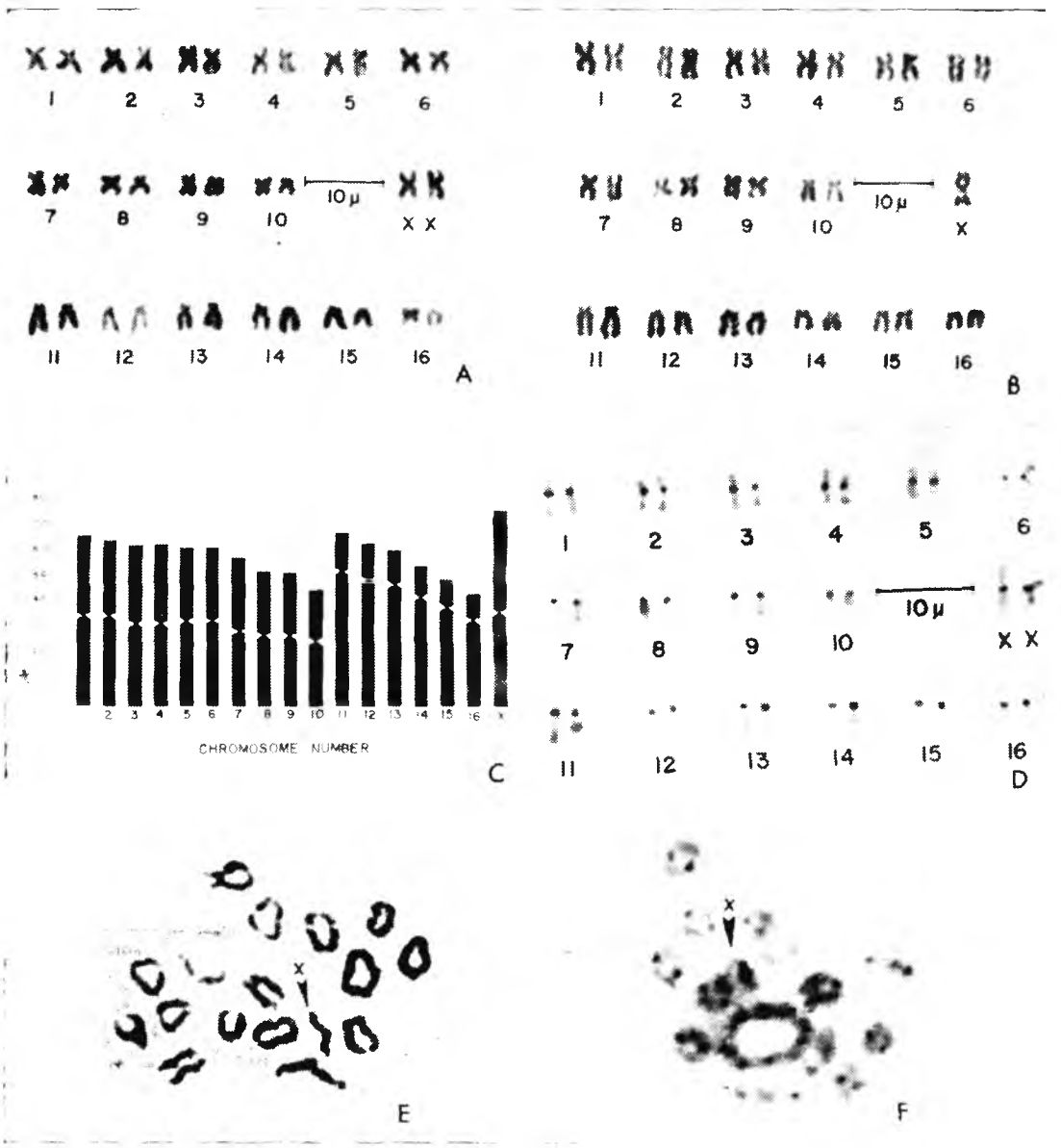

A. Female karyotype of *Periplaneta americana*; B. Male karyotype of *Periplaneta americana*; C. Idiogram of *Periplaneta americana*; D. *C*-banded karyotype of the female roach; a univalent *X*-chromosome; E. Male diakinesis showing 16 bivalents and F. *C*-banded diakinetic stage showing *C*-positive regions in the translocated ring, autosomal bivalents and the *X*-chromosome.

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KARYOTYPE ANALYSIS AND MITOTIC CYCLE OF WOOLLY APPLE APHIS (*ERIOSOMA LANIGERUM* HAUSMANN)

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The karyotype and mitotic cycle in the somatic embryonic tissues of woolly apple aphis (*Eriosoma lanigerum* Hausmann) are discussed in detail. The diploid chromosome number in this aphid species is twelve (2n=12). The chromosomes are rod-shaped and according to their length their is one pair of long, three pairs of intermediate and two pairs of short chromosomes. The karyotype of *Eriosoma lanigerum* Hausmann as reported earlier from North America is different from that observed in the present investigations possibly because of the change in the host plant and mode of reproduction.

(*Key words:* karyotype, woolly apple aphis, *Eriosoma lanigerum*)

INTRODUCTION

Woolly apple aphis (*Eriosoma lanigerum* HAUSMANN) is a serious pest of apple crop in the temperate regions of India. This insect pest is a native of North America where this aphid species undergoes cyclic parthenogenesis on elm plant (primary host) and apple plant which is the secondary host (BAKER, 1915). However, in India it has become completely adapted to apple plants and generally reproduces parthenogenetically throughout the year (RAHMAN & KHAN, 1941).

There is no mention in the literature about the detailed mitotic cell division in the somatic embryonic tissues of *Eriosoma lanigerum* HAUSMANN except its diploid chromosome number which is reported as twelve (PAGLIAI, 1963; HARPER & MACDONALD, 1966; ROBINSON & CHEN, 1969). In India, KULKARNI & KACKER (1980) also mentioned the same chromosome number but did not succeed in constructing the karyotype of this aphid species.

It is, therefore, of great interest to study the chromosomal structure and mitotic cycle in the somatic embryonic tissues of woolly apple aphis and to construct the karyotype of this insect pest which has become totally adapted to apple plants in India.

MATERIALS AND METHODS

Fresh embryos of second and third instar nymphs of woolly apple aphis were used for the study of somatic chromosomes because the cells of these embryos are mostly in dividing stages.

The embryos were pretreated with 0.7% sodium citrate solution for half an hour and then were fixed in 1:3 acetic-ethanol mixture. Slides were prepared by squash method using 5% Giemsa stain in phosphate buffer at pH 6.8 and 1% aceto-carmine stain. Photomicrographs of somatic cell division stages were taken at ×1600 magnification. Chromosomes were arranged in decreasing order of their lengths in order to construct the karyotype. The chromosomal lengths were measured by using ocular micro-meter and thus the idiogram of wooly apple aphis was prepared.

RESULTS AND DISCUSSION

During interphase stage (Fig. 1) the nucleus is enlarged and darkly stained

TABLE 1. Lengths of somatic metaphase chromosomes (Mean chromosome lengths in μm) of woolly apple aphis (*Eriosoma lanigerum* HAUSMANN)*

	Chromosome number												
	1	2	3	4	5	6	7	8	9	10	11	12	Total complement Length
Actual Length	2.91	2.57	2.14	2.02	1.93	1.87	1.73	1.70	1.61	1.37	1.33	1.10	22.28
	**±0.17	±0.12	±0.11	±0.11	±0.08	±0.09	±0.11	±0.04	±0.01	±0.10	±0.05	±0.04	±1.12
Relative Length***	13.07	11.60	9.63	9 05	8.70	8.40	7.72	7.65	7.25	6.10	5.96	4.87	
	±0.47	±0.52	±0.21	±0.12	±0.12	±0.13	±0.20	±0.20	±0.20	±0.22	±0.20	±0.33	

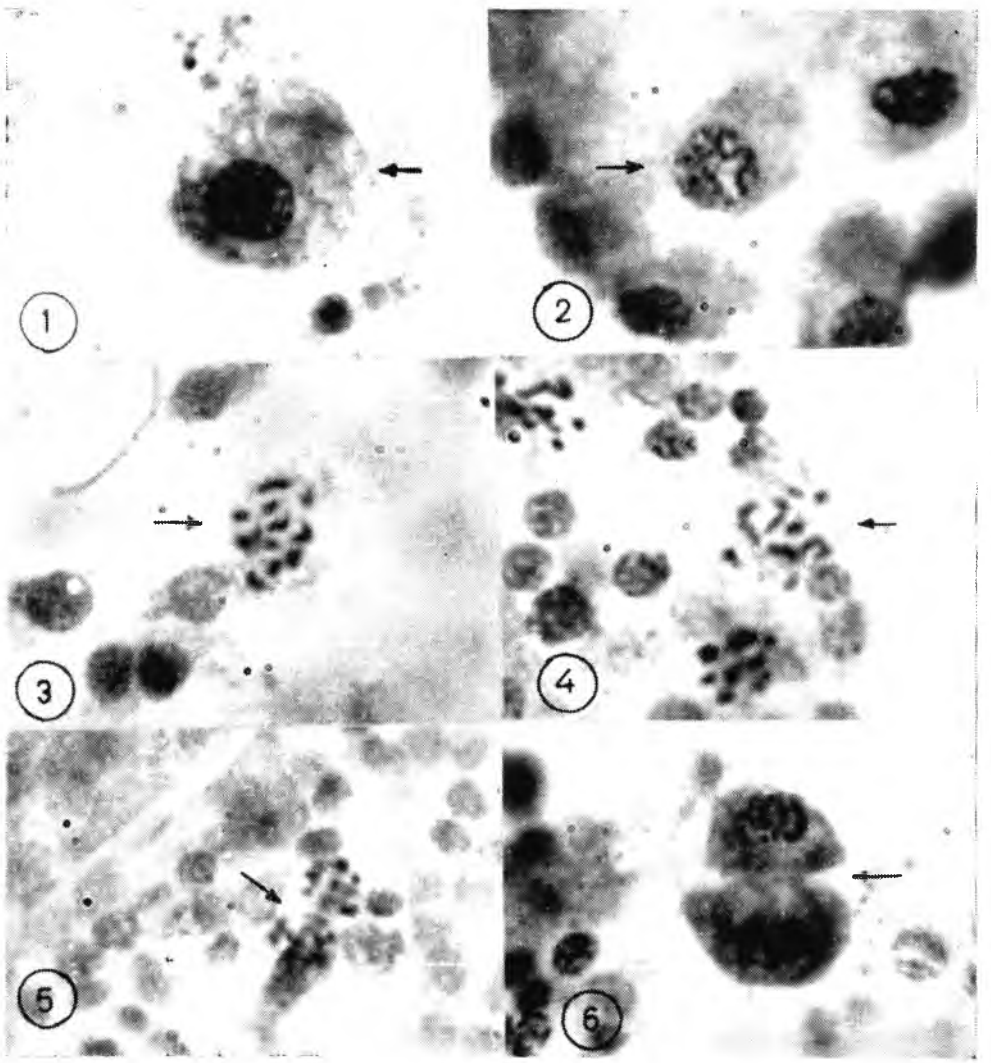
* Ten metaphase plates were selected for chromosomal measurements.
**(±) Standard error about the mean.
*** Relative length as percentage of total complement length.

while the cytoplasm is lightly stained. The chromatin threads appear thin and coiled. The nuclear membrane remains intact. The prophase (Fig. 2) is initiated when the chromosomes appear as irregu larly twisted threads. During this stage, the chromosomes start condensing but the nuclear membrane still remains intact. In late prophase, the chromosomes get shortened, however, it has not been possible to count the exact number of chromosomes in this stage.

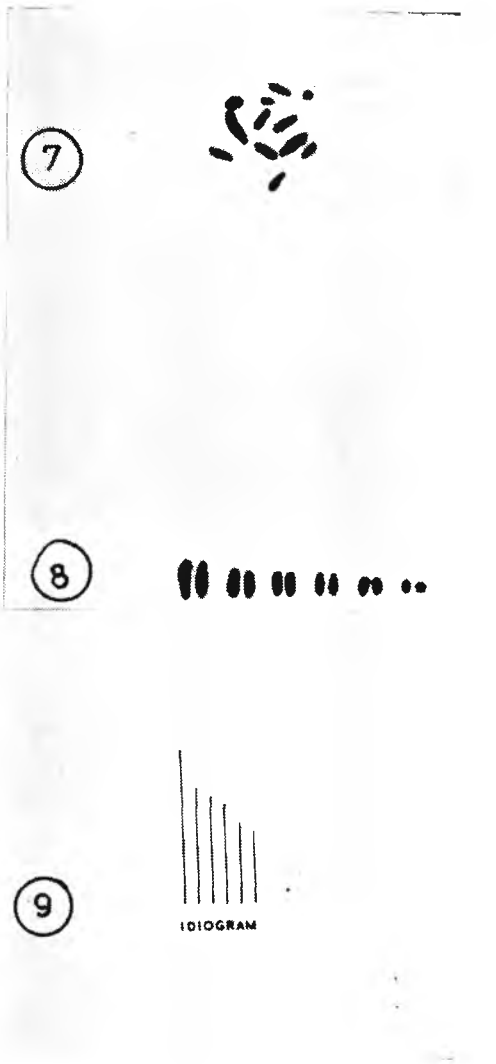
With the disappearance of nuclear membrane, the metaphase (Figs. 3, 4, 7) begins. The chromosomes are fully condensed, appear as rod-shaped and are randomly distributed. In this stage, exact counting of chromosomes can be done easily and they are twelve in number, thus confirming the earlier reports (PAGLIAI, 1963; HARPER & MACDONALD, 1966; ROBINSON & CHEN, 1969; KULKARNI & KACKER, 1980).

Ten metaphase plates (Table 1) were selected to prepare the idiogram (Fig. 9) of woolly apple aphis. The lengths of chromosomes in different metaphase plates vary possibly because they may be in different stages of development such as early and late metaphase stages as well as because of different orientations of the chromosomes. Similarly total complement length and relative length (as percentage of total complement length) of chromosomes also vary in different metaphase plates (Table 1).

Most of the chromosomes are less than 3 μm long but in some plates these may be about 4 μm. The mean actual length of chromosomes ranges from 1.10μm ±0.04 S. E. in the shortest chromosome to 2.91μm ± 0.17 S. E. in the longest chromosome. The mean total complement length is 22.28μm ± 1.12 S. E. and relative length (as percentage of total complement length) of chromosomes ranges from 4.87 ± 0.33 S. E. in the shortest chromosome to 13.07±0.47 S. E. in the longest chromosome (Table 1). According to HARPER & MACDONALD (1966) who studied the karyotype of woolly apple aphis infesting elm plants, the chromosomes



Photomicrographs of somatic cell division plates of woolly apple aphis. (Magnification ×1600)
Figs. 1. Interphase plates; 2. Prophase plate; 3 & 4 Metaphase plates; 5. Anaphase plate; 6. Telophase plate;



7. Metaphase plate; 8, Karyotype (from Fig. 7) 9. Idiogram (The lengths of the chromosomes are represented to scale, based on the measurements of the somatic metaphase plates).

may be 4.5 μm long with majority of them being under 3 μm. According to these workers the mean actual length of chromosomes ranges from 1.60μm $\pm$ 0.09 S. E. in the shortest chromosome to 3.40μm $\pm$ 0.27 S. E. in the longest chromosome. The difference between these observations may be because of the changes in the genetic make up of this aphid species which reproduces constantly by parthenogenesis on apple plants in India, whereas, in North America it undergoes a regular alternation of sexual generation with parthenogenetic generations (cyclic parthenogenesis) on elm and apple plants. Thus, the present investigations support the view of BLACKMAN (1980) that the karyotype variation within an aphid species occurs as a result of elimination of sexual generation.

Karyotype (Fig. 8) of woolly apple aphis shows that out of six pairs of chromosomes, one pair is long (2.74μm), three pairs are intermediate (2.08μm, 1.90μm and 1.72μm) and two pairs are of short lengths (1.49μm and 1.22μm).

Anaphase stage is not found so frequently like other stages possibly because it lasts for a short time. However, in 2-3 plates (Fig. 5) the chromosomal halves are seen moving towards the opposite poles. During telophase stage (Fig. 6) the chromosomal halves form compact masses at their respective poles. The nuclear membrane makes its appearance around each chromatin mass and it is followed by cytokinesis resulting in the formation of two cells.

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AUTORADIOGRAPHIC STUDIES OF THE PROTEIN METABOLISM IN THE SALIVARY GLANDS OF THE RED COTTON BUG *DYSEDRCUS KOENIGII* (PYRRHOCORIDAE : HEMIPTERA)

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The origin of salivary secretion in *Dysdercus koenigii* was investigated by autoradiography using ^{3}H-L-histidine. Short incubation of 15 min produced a heavy labelling of the double peritoneal layers, but only a moderate labelling of glandular epithelium and practically none in the lumen. With progressive increase in the incubation periods, the site of heavy labelling shifts inwards first to the glandular epithelial layer, especially to its brush border and then to the lumen wherein a falling gradient of activity from periphery inwards is perceptible. With 2 hr of incubation, labelled vesicles appear to be pinched off from the brush border and join the lumen contents. The results suggest that the glandular epithelium, besides secreting saliva, is capable of acting as a transport organ, just as the follicle epithelium investing the oocytes mediates the transport of haemolymph protein into the oocyte cortex. The possibility of saliva being produced also from precursors incorporated from the haemolymph is discussed.

(Key words: autoradiography, protein metabolism, salivary glands, *Dysdercus koenigii)*

INTRODUCTION

The salivary glands of phytophagous Hemiptera are known to produce a variety of enzymes that serve for feeding by penetration of the host plants as well as for digestion of ingested food materials (BAPTIST, 1941). Most of the syntnetic products of the principal salivary glands and the accessory glands are discharged into the plant tissues through the salivary pump (WEBER, 1928). Some components of the saliva may be utilized in *Dysdercus* for the formation of a protective sheath around the maxillary and mandibular stylets (SAXENA, 1963). The extensive studies of BAPTIST (1941) on the morphology and physiology of the salivary glands of the Heteroptera gave no evidence to show that the various lobules produce different chemical substances with varied functions. But since, the salivary glands help both in facilitating penetration and digestion, a division of labour among the lobules is suggested (see review by MILES, 1972) or possibly, the same lobule may undergo a varied programme of activity within a short period in one phase producing enzymes for piercing and sucking and in the next phase producing enzymes that hydrolyse food materials. The exact origin of salivary secretions is largely unknown, though it is tacitly assumed that all the salivary components are produced exclusively by the synthetic activity of the glandular epithelial cell.

The present report shows that the glandular epithelium of the salivary glands apart from its own synthetic activity, is

also capable of transporting materials from the haemolymph, which could be the precursors of saliva.

MATERIAL AND METHODS

Gross morphology of the salivary gland was studied with borax carmine stained whole preparations. The histology of the gland was investigated by semithin sections stained with methylene blue.

To study the protein metabolism of the salivary glands, ^{3}H-L-histidine (sp. activity: 3.1 Ci/m Mole; dosage : 5 μCi/0.05ml) was injected into the body cavity with glass needles and incubated for varying periods ranging from 16 min to 120 min. At the end of the incubation periods, the glands were dissected out and fixed in Carnoy's fluid for 2 hr. 8 μm thick deparaffinised sections of the gland were treated with cold trichloracetic acid (5%) for 15 min at 40°C and then processed for autoradiography, using Kodak AR 10 stripping film method. Exposure times varied from 3-6 weeks.

RESULT

The salivary glands of *Dysdercus koenigii* are paired compact disc-like glands of irregular shape. They show a deep groove-like depression that divides the gland into anterior and posterior halves that differ in their histological detail as well as in the histochemical behaviour. The two halves are joined by a hilus into which open a long tubular accessory gland. An exit duct leading to the salivarium also arises from the hilus (Fig. 1).

Histologically the glandular lobules are made up of a layer of cubical or columnar cells bearing conspicuous polyploid nuclei of irregular shape. The gland cells rest on a basement membrane external to which there are two peritoneal layers made up of squamous cells (Fig. 2). The inner surfaces of the glandular epithelial cells facing the spacious lumen possess the brush border (Fig. 3) which in electron micrographs is seen to bear

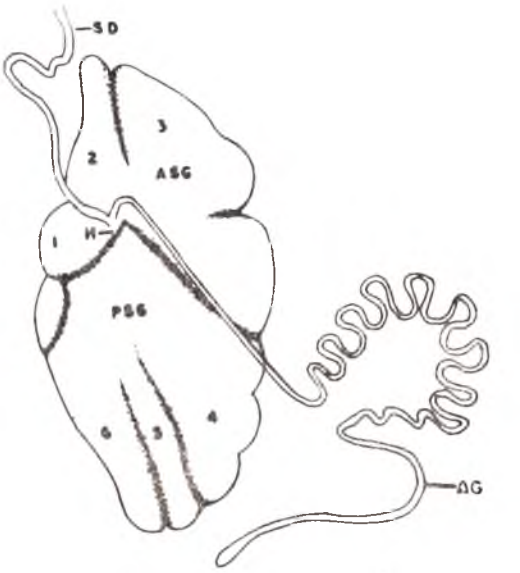


Fig. 1. Diagram of the salivary gland showing its relationship with the accessory gland (AG) and the salivary duct (SD). The principal gland is divisible into the anterior (ASG) and posterior parts (PSG) by a deep groove running across the gland and the halves are joined at the hilus (H).

numerous microvilli (unpublished observations).

In autoradiographs with 15 min incubation, the radioactivity is mostly localised in the peritoneal epithelia and the glandular epithelium of the various lobules of the gland. In the glandular lumen, very little activity is detectable and this is not much higher than the background (Fig. 4). With this incubation period, it is seen that the peritoneal layers exhibit a higher degree of labelling than the glandular epithelium (Figs. 4 and 5). The cytoplasm of the gland cells is more strongly labelled than the cell nuclei (Fig. 4). When the incubation period is prolonged to 30 min, the site of strongest labelling shifts to the glandular epithelium (Fig. 6). At the same time, the lumen contents, especially the peripheral regions

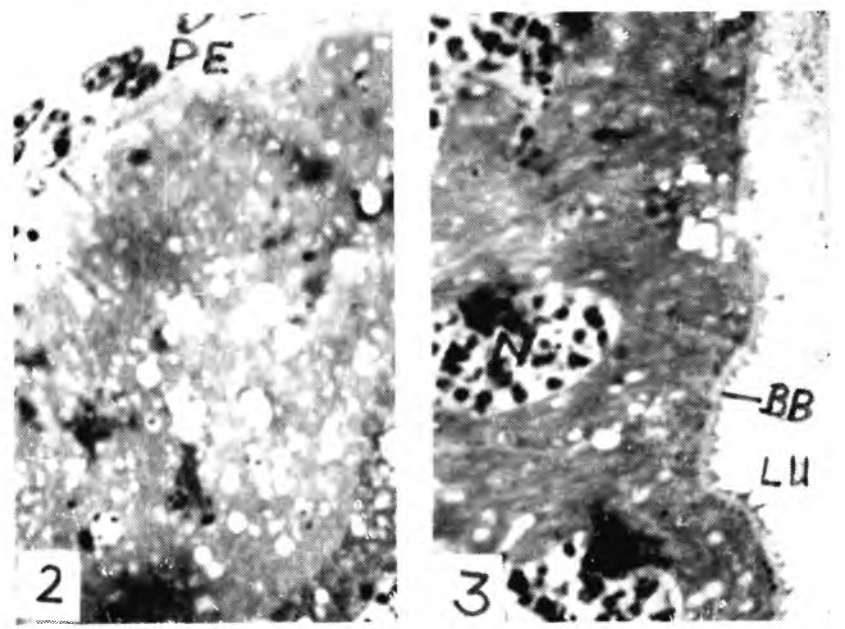
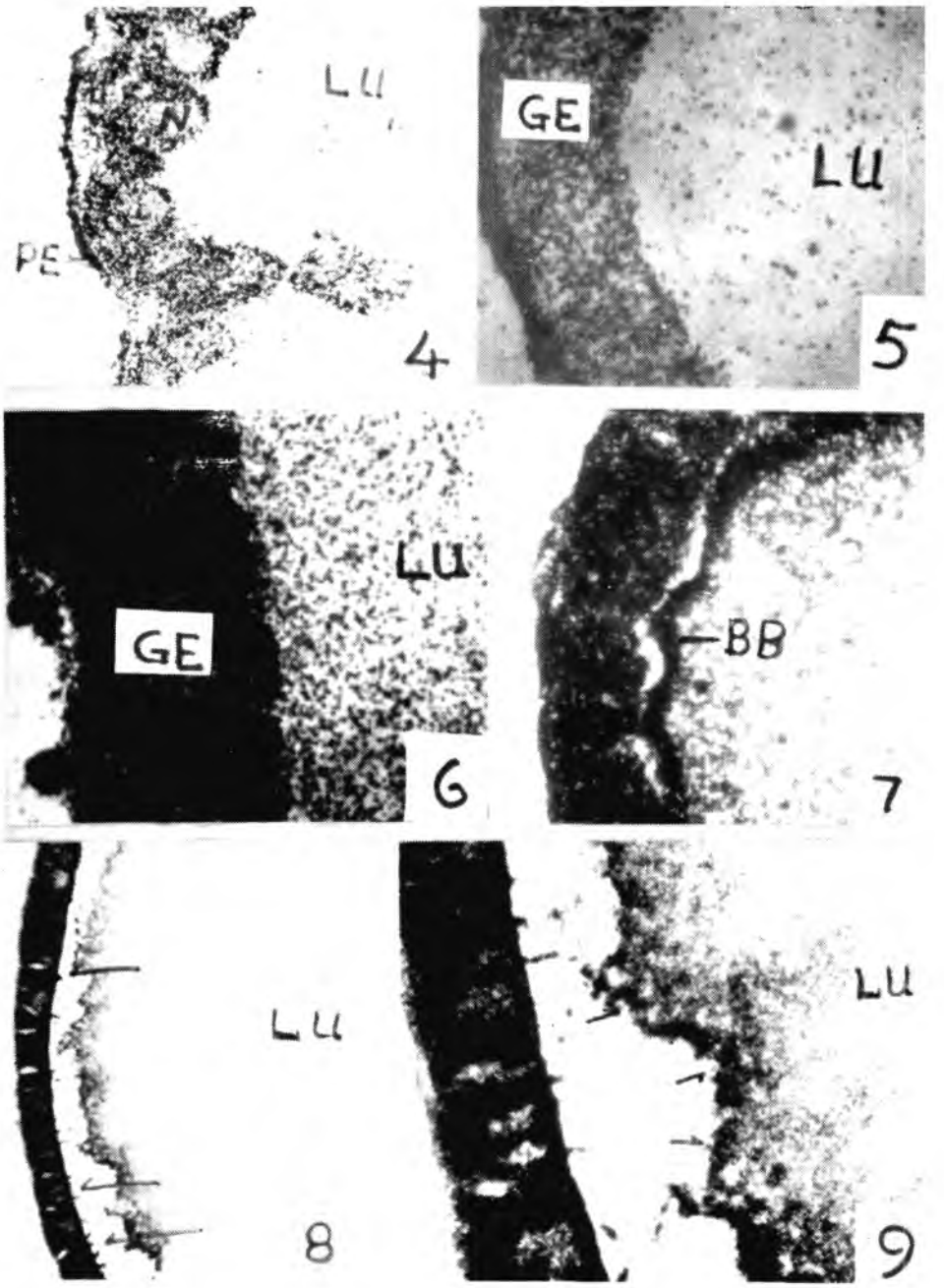


Fig. 2. Methylene blue stained semithin section of the principal gland showing its histology. The double peritoneal layers (PE), the basement membrane and the gland cell cytoplasmic area are seen. 960 ×. Fig. 3 shows the polyploid branching gland cell nuclei (N) and the brush border (BB) facing the lumen (LU) in a semithin section stained with methylene blue. 960 ×.



Figs. 4—9: Antoradiographs of the posterior half of the principal salivary glands showing the labelling patterns with ^{3}H-L-histidine in the peritoneal layers (PE), glandular epithelium (GE), brush border (BB) and the lumen (LU) with varied incubation times, magnification of all the figures is 430×, Fig. 8 is 150×. Figs. 4 & 5: 15 min incubation. The peritoneal layers show the heaviest labelling. In the gland cells the cytoplasm is more radioactive than their nuclei (N). (Fig. 4). The lumen contents show very little labelling. Fig. 6. 30 min incubation. The glandular epithelium shows heavy labelling and the lumen contents also begin to show increased radioactivity. Fig. 7. 1 hr incubation. Brush border shows heavy labelling. Labelled vesicles (⟶) begin to appear in the lumen. The lumen contents exhibit a gradient of activity falling from periphery inwards. Figs. 8 & 9. 2 hr incubation. Gradient of activity in the lumen contents becomes intensified. Release of several vesicles (⟶) from the brush border (Fig. 8) and their merger with the peripheral parts of the lumen contents (⟶) is seen. The vesicles soon lose their identity in the lumen.

begin to show a level of radioactivity which is distinctly higher than the background (Fig. 6). With an incubation period of 1 hr, there is a general increase in the intensity of labelling both in the glandular epithelial layer as well as in the lumen. Especially the brush border shows a higher density of silver grains than the epithelial cells (Fig. 7). The lumen contents also show an increased radioactivity, wherein a gradient falling from periphery inwards is detectable (Fig. 7). Here and there labelled vesicles become perceptible in the periphery of the lumen. When the incubation time is extended to 2 hr, it is seen that the peritoneal layers and the glandular epithelium show a somewhat diminished level of labelling as compared with the lumen contents, especially in its peripheral parts, because of the exhaustion of the available pool of labelled molecules. With still longer incubation periods, this feature becomes more accentuated and there is a progressive increase of labelled material in the lumen. The gradient of activity observed with 1 hr incubation, is still more intensified. Regions of the lumen close to the brush border are more radioactive than the deeper regions of the lumen (Figs. 8 and 9). Furthermore, a large number of labelled vesicles seem to be liberated from the brushborder of the glandular cells into the lumen where they become integrated into the peripheral parts of the lumen contents, thereby increasing the level of labelling at this site (Figs. 8 and 9). These vesicles however do not tend to remain as such and accumulate in large number. On the contrary, they seem to be rapidly turned over or otherwise modified after they join the lumen contents, so that they are no longer detectable as labelled vesicles in the deeper parts of the lumen.

It should, however, be pointed out that the labelling pattern in all parts of the glandular lobules is not uniform. The above observations relate to the posterior half of the principal gland The anterior parts of the gland seem to be not very much involved in the transport activity, as seen from their labelling patterns.

DISCUSSION

These observations demonstrate that the labelled molecules move from the haemolymph across the peritoneal layers the basement membrane and the glandular epithelium to become incorporated into the lumen. The shifting of the site of heavy labelling from outside inwards with progressive increase in incubation periods namely, from the peritoneal layers to the glandular epithelium and then to the brush border and finally to the lumen, where again a gradient of falling activity is clearly manifest, indicates that the salivary glands are capable of transporting materials from the haemolymph into the lumen (KUMAR, 1979). According to SCHIN & CLEVER (1968) even foreign proteins, such as human serum albumin and ferritin, are capable of entering the salivary gland cells, but these exogenous materials were shown to enter the lysosomes of the gland cells, which indicates that they may not be transported as intact molecules into the lumen, but degraded first. However, in *Chironomus* (LAUFER & NAKASE 1965), the secretory proteins in the gland cells were reported to exhibit the same immunological properties as those of the blood proteins and hence the glandular epithelium was regarded as a transport organ for haemolymph proteins. The amino acids injected into the haemolymph have appeared in the watery saliva of the plant bugs and this was

interpreted as the excretory activity of the salivary apparatus (MILES, 1967a).

Our histochemical analyses of the lumen contents showed that they can be characterised mainly as ribonucleoproteins with a component of glycogen and lipids. Besides, proteases and peroxidase could be demonstrated. The brush border is specially an active site for the incorporation of both the exogenous materials as well as for the discharge of the synthetic products of the gland cells. This is characterised by the existence of a large number of microvilli which facilitate the incorporation of materials by a process akin to micropinocytosis (unpublished EM observations). The EM studies on the salivary glands of aphids by MOERICKE & WOLFARTH-BOTTERMANN (1960) showed that the secretion products accumulate in membrane bound vesicles which later break down to release their contents into the lumen. Our autoradiographs show a corresponding process of vesicle formation (Figs. 8 and 9).

The mode of transport of materials from the haemolymph into the salivary glands and the cytoarchitecture that facilitates this process, show a great deal of similarity to the manner of haemolymph yolk protein incorporation into the developing oocytes through the mediation of the follicle epithelium, as reported by the autoradiographic studies of a number of polytrophic insect ovaries (BIER, 1962; RAMAMURTY, 1964; ANDERSON, 1971; ENGELS & RAMAMURTY, 1976; see also reviews by TELFER, 1965; ENGELMANN, 1970). The mechanism of incorporation of yolk protein into the oocytes is by pinocytosis, as shown by EM studies of a variety of insect species (KESSEL & BEAMS, 1963; ROTH & PORTER, 1964; ANDERSON, 1964; BIER & RAMAMURTY, 1964).

Besides the female specific yolk protein (vitellogenin), it is well known that there are other non-vitellogenic protein fractions as well as free amino acids in the insect blood (see reviews by WYATT, 1961; CHEN, 1966). In the present species, it remains to be proved that the material incorporated into the glandular lumen, as seen in the autoradiographs, has the same immunological properties as the non-vitellogenic haemolymph protein fractions. In the absence of such evidence, it cannot be concluded that what is seen to be moving across the glandular epithelium is the blood protein. Nevertheless, from the transport phenomena demonstrated here, the possibility cannot be excluded that one or more of these non-vitellogenic blood protein fractions may be utilized by the salivary glands as the precursors of saliva.

The lack of uniformity of labelling pattern in the anterior and posterior halves of the principal gland observed here, suggests that the different glandular lobules synthesise proteins (enzymes) at different rates and that they incorporate the haemolymph materials in varied degree. Presumably this is due to differences in the functional activities of the different component parts of the salivary apparatus, which is suggestive of a possible division of labour among the various glandular lobules, as proposed by MILES (1967a).

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EFFECT OF THIOUREA ON LIPID SYNTHESIS IN THE OVARIOLES OF FLESH-FLY, *SARCOPHAGA RUFICORNIS* (FABR.) (DIPTERA : CYCLORRHAPHA)

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In normal *Sarcophaga* the cytoplasm of both the follicular epithelial cells and the nurse cells appear to have evenly distributed lipid in them. Their nuclei are also sudanophilic. The ooplasm as well as oocyte nucleus give strong positive reaction for lipid. In 0.5% thiourea administered *Sarcophaga* the cytoplasm as well as the nuclei of follicular epithelial cells and nurse cells stain faintly with Sudan Black B indicating marked reduction in their content. The ooplasm along with the oocyte nucleus also do not get stained deeply which clearly points out that thiourea affects the lipid deposition in the ovarioles.

(Key words: Sarcophaga ruficornis, ovarioles thiourea, lipid synthesis).

INTRODUCTION

Lipids have been reported to be one of the main sources of energy for the developing embryo (GILBY, 1965). Mature eggs of many species have been found to contain large amount of lipids (ENGELMANN, 1970). LAUGHLIN (1956) has noted that during the larval stages the lipid content increases due to absorption or synthesis. It is generally suspected that the Golgi elements play an important role in the synthesis of lipids. So far very little work has been done on the effect of chemosterilants on lipid synthesis in insect eggs. In the present paper an attempt has been made to study the effect of thiourea on synthesis of lipid in the ovarioles of flesh fly, *Sarcophaga ruficornis* (FABR.).

MATERIALS AND METHODS

Sudan Black B technique (PEARSE, 1968) was used to visualise lipid in *Sarcophaga* ovary. Newly emerged flies obtained from the laboratory culture were divided into two groups. One group was fed with the diet (bread soaked with milk) containing 0.5% thiourea whereas others, used as control, were given the normal food throughout the observation period. Both the control, and experimental flies, were dissected in Ringer's solution on 4th and 6th day of emergence. Their ovaries were taken out and were fixed in formol calcium for 20 hours at room temperature. After fixation, the ovaries were dehydrated, cleared and embedded in paraffin wax in usual manner. 8 μm thick paraffin sections were stained in saturated Sudan Black B solution for 30 minutes. The stained slides were rinsed quickly in 70% alcohol. Then after giving a dip in distilled water they were mounted in glycerine jelly.

RESULTS AND DISCUSSION

Sarcophaga ovariole was found to show the lipid material in abundance and widely distributed when stained with Sudan Black B. The cytoplasm of the follicular epithelial cells appears to be strongly sudanophilic and is stained intensely. Their nuclei also gave strong positive reaction. The nurse cell cytoplasm stains deeply, and the lipid appears to be evenly distributed. The nurse cell nucleus is also stained with the Sudan Black B (Fig. 1).

In oocyte it was observed that the sudanophilic lipid content is directly proportional to the age of the fly. In younger oocyte, the ooplasm is less sudanophilic in comparison to the older ones. In older individuals the lipid content gradually increases (Fig. 2). Under high magnification it was observed that the coarse sudanophilic bodies are dispersed throughout the ooplasm between the large yolk bodies. The most interesting observation was that the peripheral region of the ooplasm, lying near the follicular epithelial cells and the nurse cells stains darkly and is conspicuously sudanophilic. This gives an impression that the lipid molecules are probably being contributed by the follicular epithelial cells and the nurse cells (Fig. 1). BONHAG (1955) has made similar observations on *Oncopeltus fasciatus*. According to him both the follicular epithelial cells and the nurse cells contribute lipid to the developing oocyte. In *Drosophila* also, the nurse cells have been reported to supply lipid to the developing oocyte (HSU, 1953; FALK & KING, 1964).

Unlike the normal flies, the ovarioles of 0.5% thiourea treated individuals do not get stained deeply with Sudan Black B which clearly points out that thiourea affects the lipid deposition in the ovariole (Figs. 3, 4). Similarly, the nuclei of the follicular epithelial cells also show no reaction with Sudan Black B. However their cytoplasm stains faintly. The nurse cell cytoplasm stains uniformly with the dye. Their nuclei also give sudanophilic reaction. The ooplasm is also stained with Sudan Black B. The oocyte nucleus, however, stains faintly. The most interesting observation was that unlike the normal flies, in treated individuals, the margin of ooplasm adjacent to the follicular epithelial cells and the nurse cells do not stain dark. This clearly indicates that thiourea, besides affecting the lipid synthesising capacity of the ovariole components also hampers the supply of lipid from them to the developing oocyte.

Earlier studies on *Sarcophaga* reveals that thiourea-treated ovarioles show marked reduction in their DNA (TRIPATHI & CHAUDHRY, 1980); RNA (TRIPATHI & CHAUDHRY, 1979) and protein (TRIPATHI, 1981) contents as well as complete lack of glycogen (TRIPATHI & CHAUDHRY, 1979) deposition. It appears, therefore, that due to absence of raw materials like protein, lipid and carbohydrate, the oocyte of thiourea fed flies fails completely to synthesise yolk (CHAUDHRY & TRIPATHI, 1976). Consequently the egg does not develop properly.

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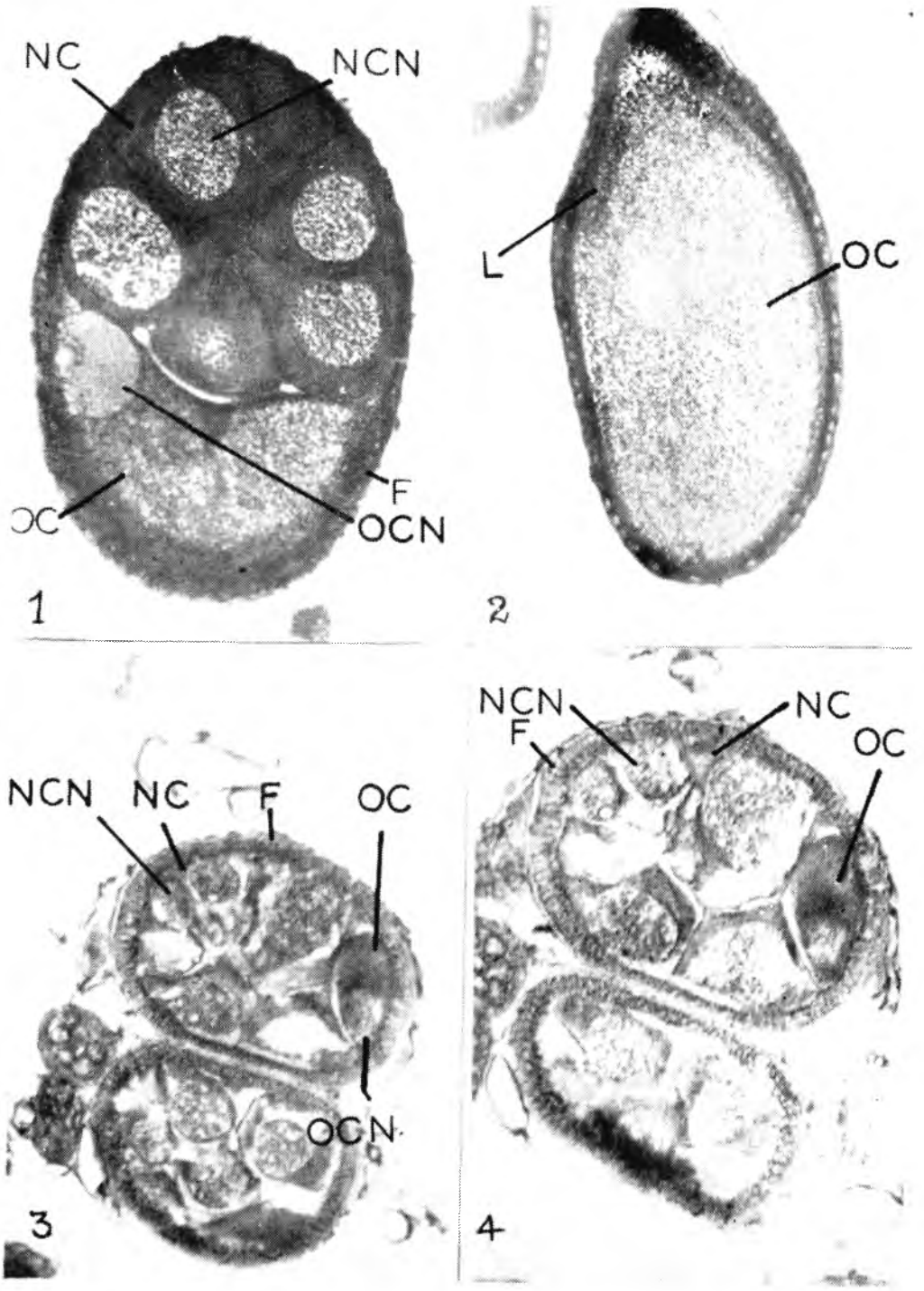

Fig. 1—Section of 96 hours old ovary of normal *Sarcophaga ruficornis* × 200; Fig. 2—Section of 144 hours old ovary of normal *Sarcophaga ruficornis* × 200; Fig. 3—Section of 96 hours old ovary of 0.5% thiourea treated *Sarcophaga ruficornis* × 200; Fig. 4—Section of 144 hours old ovary of 0.5% thiourea treated *Sarcophaga ruficornis* (Fabr.) × 200. F—Follicular epithelium; NC—Nurse cell; NCN—Nurse cell nucleus; OC—Oocyte; OCN—Oocyte nucleus; L—Lipid.

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CHELAL GROWTH IN THREE SPECIES OF SOUTH INDIAN PSEUDOSCORPIONS AND THEIR TAXONOMIC RELEVANCE

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The variation in the position of trichobothria of chelal fingers in adult pseudoscorpions are of taxonomic importance. Their stabilization in post-embryonic stages exhibit relative growing regions on the chelal axis in closely allied forms-*Paratemnus indicus* Sivaraman and *Tamenus indicus* Sivaraman (Family Atemnidae) than *Tullgrenius indicus* Chamberlin (Family Miratemnidae). The relationships of the two families are discussed based on the stabilization of trichobothria and the concentration of growing region along the chelal axis.

(Key words: disposition of trichobothria, Atemnidae and Miratemnidae growth, taxonomic importance)

INTRODUCTION

The position of the named trichobothria or tactile setae on the chelal fingers brings out variation in taxa of pseudoscorpions. These variations are due to the differences in their stablization along the chelal axis during post-embryonic development. Gabbut (1965—72) in his analyses on the successive stages of British species of families Neobisiidae and Chernetidae elucidates the existence of a linear relationship in their successive stabilization. Vachon (1934, 1943), Morikawa (1962), Gabbutt & Vachon (1965) and Di Castri (1965) in their work on chelal growth pattern, emphasised its taxonomic relevance.

Dumitresco *et al.* (1970) have elevated the families Atemnidae and Miratemnidae from sub-family levels (previously regarded as sub-families Atemninae and Miratemninae of family Atemnidae) based on their study. The study on the disposition of trichobothria and concentration of growing region along the chelal fingers has been taken up presently in three species-*Paratemnus indicus* Sivaraman, *Tamenus indicus* Sivaraman (Atemnidae) and *Tullgrenius indicus* Chamberlin (Miratemnidae), to assess the taxonomic status of the same.

MATERIALS AND METHODS

Paratemnus indicus (from Pondicherry), *Tamenus indicus* and *Tullgrenius indicus* (both from Madras) have been collected in large number (both nymphs and adults) from barks of trees. The cleared and mounted specimens were taken for analyses. The chelae mounted with exterior or lateral side uppermost were used for the measurement of chelal axis and placement of named trichobothria as suggested by Gabbutt and Vachon (1965).

Measurement of chelal axis:

The distance from the tip of the movable and fixed fingers to the anterior edge of the condylar outgrowth of the palm are measured as chelal axis (Gabbutt & Vachon, 1965).

Measurement of named trichobothria:

The movable finger of chela carries one series of four trichobothria (*t*-terminal, *st*-subterminal, *sb*-subbasal and *b*-basal); the fixed finger carries two series of four trichobothria as an external series (*et*-exterior terminal, *est*-exterior sub terminal, *esb*-exterior sub basal and *eb*-exterior basal) and an internal series (*it*-interior terminal, *ist*-interior sub terminal, *isb*-interior sub basal, *ib*-interior basal. Vachon

(1936) showed, of the twelve trichobothria on the chela of the adult, the tritonymphs possess ten, the deutonymphs eight and the protonymphs four.

The disposition of all the trichobothria during the course of post-embryonic development are measured along the chelal axis, as the distance from the tip of the respective finger to the arolium of trichobothrium.

Measurements (in mm) of chelal fingers of the three species were made (sample size = 30) along the chelal axis and the position of named trichobothria marked.

OBSERVATIONS AND DISCUSSION

Successive positions of Trichobothria:

The stabilization of trichobothria vary in the three species during post-embryonic development (Table 1). The relative distance and sequence of the named trichobothria also vary among them (Table 2). In *Paratemnus indicus* and *Tamenus indicus* *sb* in the movable finger and *Ist* in the fixed finger are last to be stabilized in adult, whereas in *Tullgrenius indicus* *sb* and *it* are stabilized last. The relative positions of the trichobothria along the chelal fingers of the three species are shown in Figs. 1, 2 and 3.

In both atemnid genera (*Paratemnus* and *Tamenus*), *est* is much proximal to *it; esb* proximal to *isb; b* at the basal position of chelal axis, and *ist* is last to be stabilized. In miratemnid genus (*Tullgrenius*) *est* is distal to *it; esb* distal to *isb; eb* at the basal position of chelal axis, and *it* is last to be stabilized.

The length of the chelal axis increases at each stage (Table 3) and the average increment (mm) of the chelal axis of successive stages of development vary based on the growth achieved at each stage (Table 4). The increment is more in nymphal development in atemnids and in miratemnid during tritonymph to adult transformation. It is evident that *Paratemnus* and *Tamenus* possess shorter chelal axes in protonymph stage and gain higher

TABLE 1. Trichobothrial formula and appearance of trichobothria in post-embryonic stages and adult of three species.

Species	chelal finger	protonymph	deutonymph	tritonymph	adult
Paratemnus	movable finger	*t*	*b*	*st*	*sb*
indicus Sivaraman	fixed finger exterior series	*et, eb*	*est, esb*	—	—
	fixed finger interior series	*ib*	*it*	*isb*	*ist*
Tamenus	movable finger	*t*	*b*	*st*	*sb*
indicus Sivaraman	fixed finger exterior series	*et, eb*	*est*	*esb*	—
	fixed finger interior series	*ib*	*it, isb*	—	*ist*
Tullgrenius	movable finger	*t*	*b*	*st*	*sb*
indicus Chamberlin	fixed finger exterior series	*et, eb*	*est*	*esb*	—
	fixed finger interior series	*isb*	*ist, ib*	—	*it*

TABLE 2. The distance of named trichobothria from the distal end of the chelal axis of the movable and fixed fingers are given for the Protonymph (values of a_0), Deutonymph (values of a_1), Tritonymph (values of a_2), Male (values of a_3) and Female (values of a_4).

Species	et	it	est	ist	t	st	isb	esb	ib	sb	eb	b
Paratemnus indicus												
P a_0	0.1936	—	—	—	0.4725	—	—	—	0.5041	—	0.5354	—
D a_1	0.2349	0.3888	0.4671	—	0.5346	—	—	0.7398	0.8208	—	0.8640	0.8235
T a_2	0.3429	0.5778	0.8748	—	0.9450	1.2528	1.1178	1.2208	1.1826	—	1.3176	1.8136
♂ a_3	0.3780	0.7201	0.9990	0.9450	1.0711	1.4399	1.4850	1.5884	1.5209	1.6065	1.6289	1.8136
♀ a_4	0.4139	0.7471	1.0079	0.9450	1.0665	1.3818	1.4761	1.5390	1.6289	1.9660	1.6829	1.8271
Tamenus inideus												
P a_0	0.1674	—	--	—	0.4104	—	—	—	0.3996	—	0.4401	—
D a_1	0.1674	0.2430	0.3051	—	0.4563	—	0.5454	—	0.6183	—	0.6615	0.7290
T a_2	0.1863	0.2484	0.3510	—	0.5238	0.7803	0.7236	0.7803	0.8397	—	0.9126	1.0530
♂ a_3	0.2268	0.3510	0.4914	0.5670	0.6804	1.0152	1.1286	1.2177	1.2528	1.4580	1.3635	1.6632
♀ a_4	0.2214	0.3834	0.5778	0.6264	0.6318	0.9828	1.1232	1.2933	1.3203	1.4094	1.4499	1.6416
Tullgrenius indicus												
P a_0	0.3078	—	—	—	0.6156	--	0.7182	—	—	—	0.8370	—
D a_1	0.2839	—	0.6480	0.7479	0.6642	—	0.9342	—	0.9612	—	0.9936	0.8937
T a_2	0.2889	—	0.7074	0.8640	0.6642	0.9153	1.0638	0.9855	1.1205	—	1.1583	1.0746
♂ a_3	0.3510	1.0962	0.9747	1.2636	0.7560	1.2825	1.4553	1.3959	1.5390	1.3176	1.5660	1.4580
♀ a_4	0.3483	1.1718	1.0179	1.3500	0.8964	1.2609	1.5444	1.4580	1.6416	1.2798	1.6686	1.5444

TABLE 3. The average increment (mm) calculated along the chelal axis of successive stages.

Paratemnus indicus:					
Protonymph	→	Deutonymph	= 1.1394 − 0.8146	=	0.3248
Deutonymph	→	Tritonymph	= 1.8306 − 1.1394	=	0.6912
Tritonymph	→	Male	= 2.2499 − 1.8306	=	0.4193
Tritonymph	→	Female	= 2.4929 − 1.8306	=	0.6623
Total = Protonymph	→	Male	= 2.2499 − 0.8146	=	1.4353
Total = Protonymph	→	Female	= 2.4929 − 0.8146	=	1.6783
Tamenus indicus:					
Protonymph	→	Deutonymph	= 0.9612 − 0.729	=	0.2322
Deutonymph	→	Tritonymph	= 1.3122 − 0.9612	=	0.351
Tritonymph	→	Male	= 2.0412 − 1.3122	=	0.729
Trltonymph	→	Female	= 2.1114 − 1.3122	=	0.7992
Total = Protonymph	→	Male	= 2.0412 − 0.729	=	1.3122
Total = Protonymph	→	Female	= 2.1114 − 0.729	=	1.3824
Tullgrenius indicus:					
Protonymph	→	Deutonymph	= 1.2204 − 1.0476	=	0.1728
Deutonymph	→	Tritonymph	= 1.4013 − 1.2204	=	0.1809
Tritonymph	→	Male	= 1.8306 − 1.4013	=	0.4293
Tritonymph	→	Female	= 2.025 − 1.4013	=	0.6237
Total = Protonymph	→	Male	= 1.8306 − 1.0476	=	0.783
Total = Protonymph	→	Female	= 2.025 − 1.0476	=	0.9774

growth than *Tullgrenius*, which possesses comparatively longer chelal axis in protonymph and gain moderate growth during post-embryonic development.

Figs. 4, 5 & 6 show the relative position of the trichobothria along their chelal axes and the growing region which occupies approximately the same position in subsequent stages of development. The growth zone is concentrated in the middle of the fingers and subsequently the distal setae become more distal and proximal setae become more proximal, progressively in all species. In *P. indicus* (Fig. 5) and *Tamenus indicus* (Fig. 6) the growth is concentrated between *est* and *isb*, and in *Tullgrenius indicus* (Fig. 7) between *et* and *ist*. The shift of the zone of maximum growth towards the distal half of the finger in latter accounts for the clustering of setae in the proximal half of the fingers. Similar growth phases are noticed in movable fingers also in all the three species.

Beier (1932) distinguishes Atemninae and Miratemninae as the subfamilies of Family Atemnidae Chamberlin based on the position of the pseudotactile seta on the tarsus of IV walking leg, in the former at the base and in the latter proximal to middle of tarsus. But Dumitresco *et al.* (1970) elevated the status of subfamily Miratemninae as family Miratemnidae based on the clustering of trichobothria at the proximal half of the finger in the two known miratemnid genera-*Diplotemnus* and *Tullgrenius*. Muchmore (1975) has

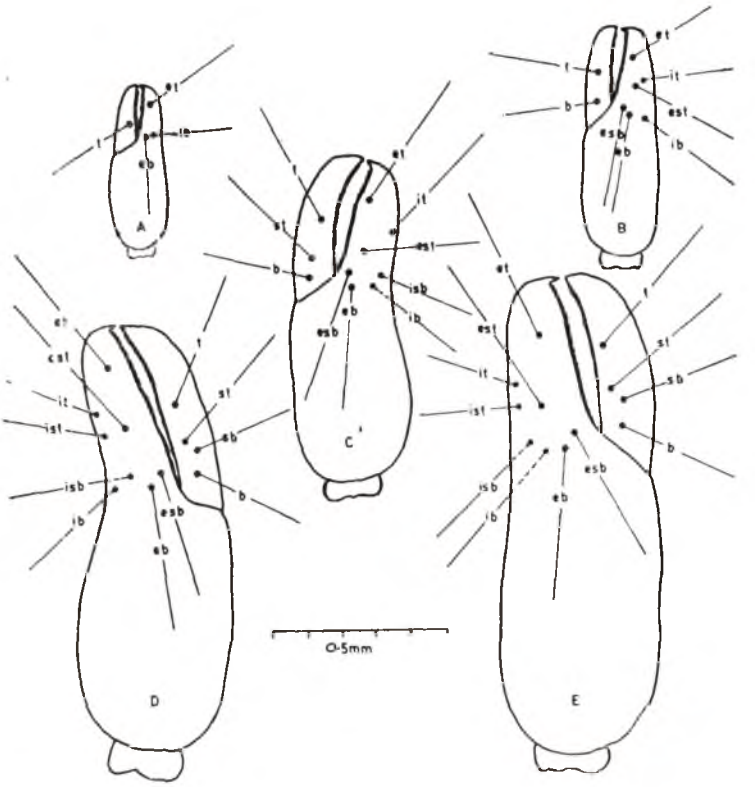


Figure 1. Chelae of *Paratemnus indicus*, A—protonymph, B—deutonymph, C—tritonymph, D—male and E—female.

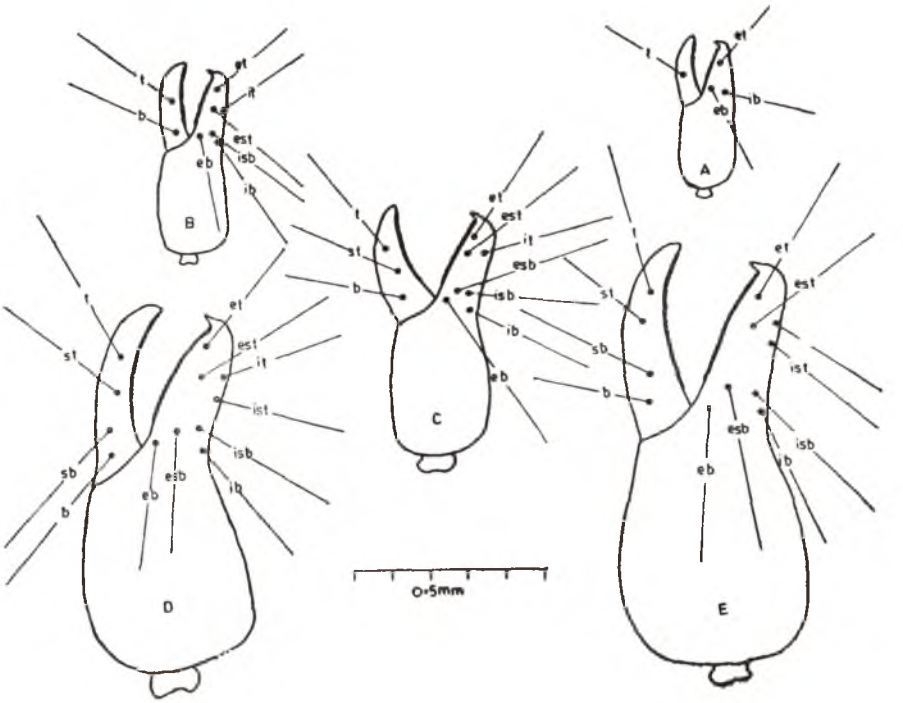


Fig. 2. Chelae of *Tamenus indicus*, A· protonymph, B—deutonymph, C—tritonymph, D—male and E—Female.

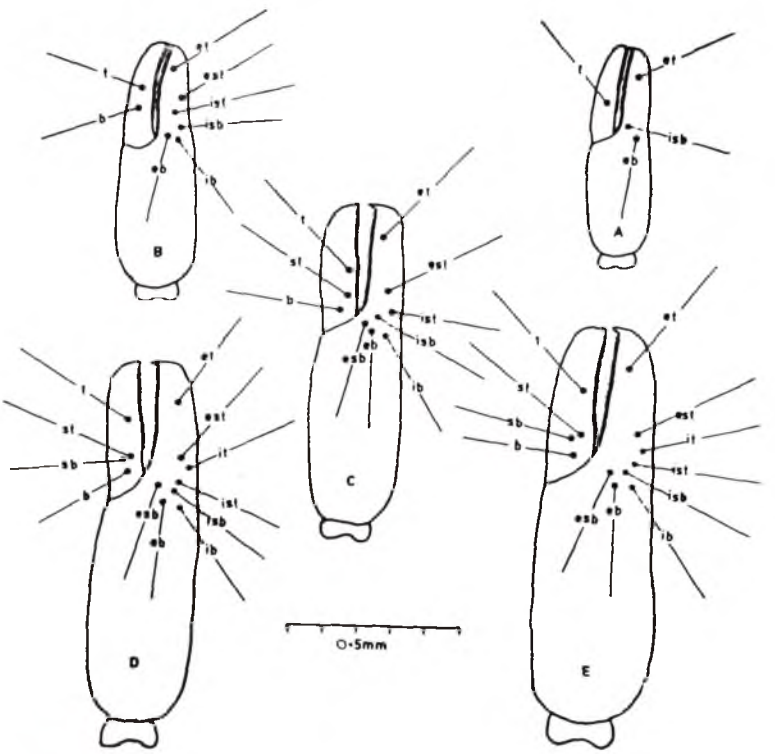
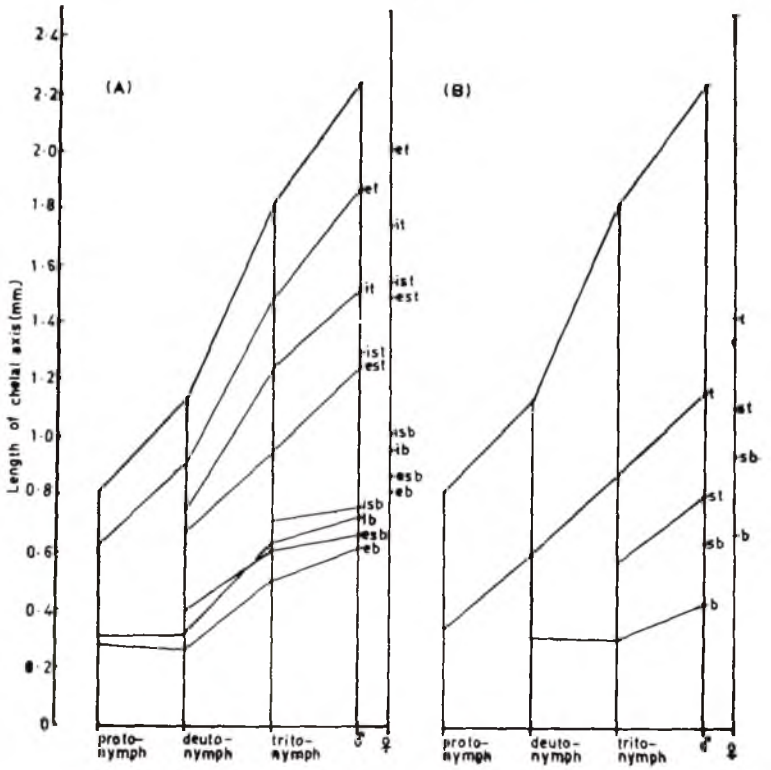


Fig.3 Chelae of *Tullgrenius indicus*, A—protonymph, B—deutonymph, C—tritonymph, D—male and E—female.



Figs. 4, 5 & 6: Named trichobothria are plottted in their sequence on the chelal axis (mm) of A-fixed and B-movable fingers of the protohymph deutonymph, tritonymph and male (female), to show their stabilization during post-embryonic development. Fig. 4. *Paratemus indicus*

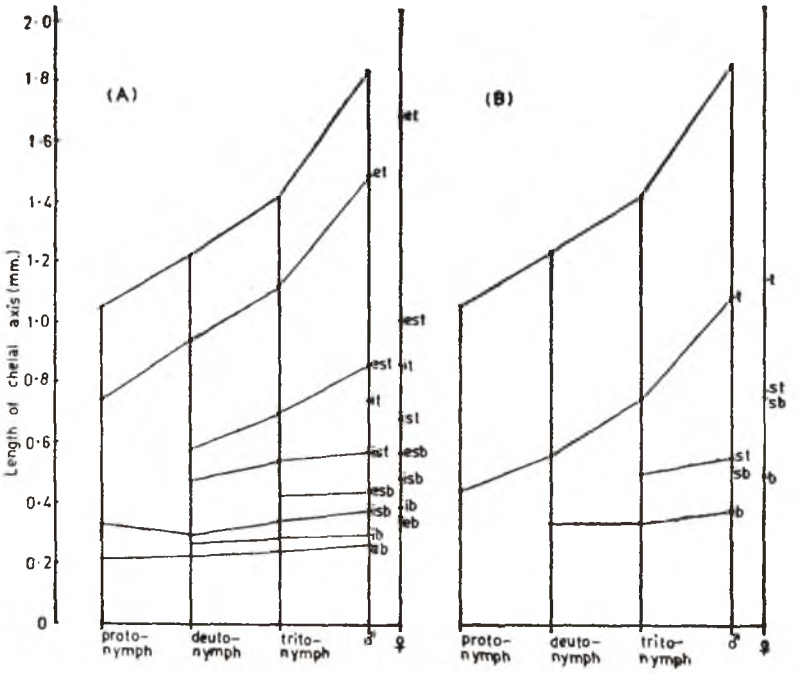


Fig. 5. *Tamenus indicus*

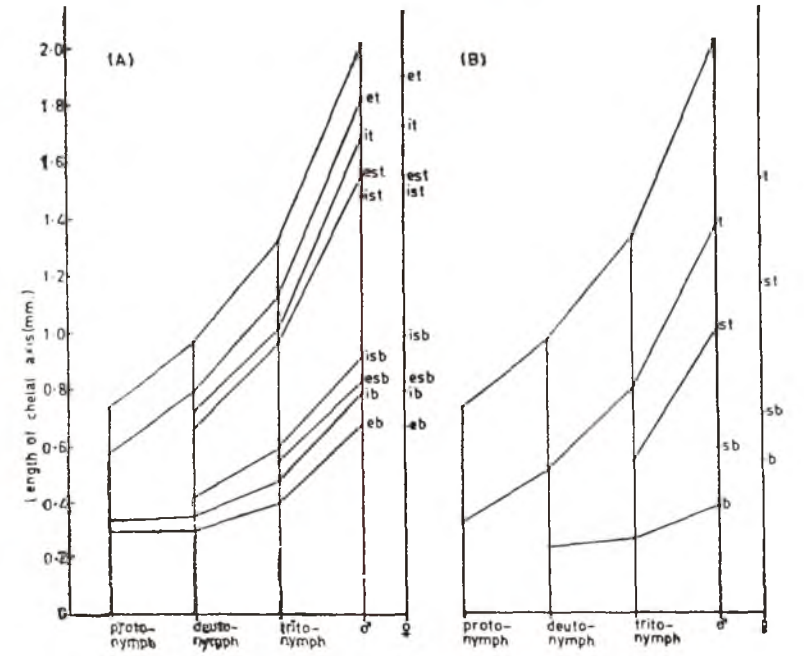


Fig. 6. *Tullgrenius indicus*

also emphasised the importance of elevating the level of Miratemnide, based on his new genus, and some new species of *Diplotemnus*. The present analysis on the stabilization of trichobothria and orientation of zone of maximum growth (along the proximal or distal regions of the chelal fingers) in atemnid and miratemnid pseudoscorpions clearly indicates their distinction in the following features: *ist* in atemnids and *it* in miratemnids are the last setae to be stabilized in adults; *esb* proximal to *isb* in atemnids and distal to *sb* in miratemnids; *ib* in atemnids and *isb* in miratemnid are initially stabilized in protonymph; the orientation of zone of maximum growth towards middle in atemnids and towards distal in miratemnid resulting in clustering of setae at the proximal portion of fingers in latter; and the average increment of growth is more during nymphal transformation in atemnids and during tritonymph to adult transformation in miratemnid. The quantitative differentiation based on the growing and non-growing regions of the chelal axis and proximal displacement of centre of growing regions of this study are to be published elsewhere. Similar studies on the related genera of Atemnidae and Miratemnidae may further confirm the taxonomic status of the respective families.

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SOME OBSERVATIONS ON POTATO CUTWORMS AND THEIR NATURAL ENEMIES

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Studies made on the occurrence and natural enemies of potato cutworms at Simla, Jullundhur, Patna and Rasalpur (Karnataka) showed that *Agrotis ipsilon* (Hfn.) was a serious pest of potato only in Patna and Jullundhur, *A. segetum* (D & S) on Simla hills, *A. spinifera* Hb. in Rasalpur, *A. flammatra* Fabr. in Patna and *Xestia c-nigrum* (L.) on Simla hills. Five parasitoids viz, *Macrocentrus collaris* (Spin.), *Netelia ocellaris* (Thomson) sp. complex, *Coelichneumon* sp. nr. *truncatulus* Thomson, *Periscepsia carbonaria* Panzer and *Turanogonia chinensis* Wiedemann, a digger wasp, *Ammophila* sp. and an entomogenous fungus, *Metarrhizium anisoplae* (Metch.) Soropin were recorded on *A. segetum* at Simla; the entomophilic nematode, *Neoaplectana* sp. was observed parasitizing the caterpillars at Jullundhur and Rasalpur. *C. truncatulus*, *P. carbonaria* and *T. chinensis* were reared at Jullundhur from *A. ipsilon* and *A. segetum*. *Apanteles ruficrus* Hal. attacked *A. ipsilon* in Jullundhur and Patna. *A. spinifera* and *A. segetum* were parasitized by *N. ocellaris*, *T. chinensis*, *Enicosphilus* sp. and *Neoaplectana* sp. in Rasalpur in Kodagu.

(Key words: Potato cutworms, *Agrotis* spp., natural enemies)

INTRODUCTION

The identity of cutworms attacking potato in India and of their natural enemies has received scant attention in India. As studies on the species composition of cutworms and their natural enemies were considered important for the effective management of the pests, the present investigations were carried out in Simla 31.06°N (Himachal Pradesh), Jullundhur 31.19°N (Punjab), Patna 25.37°N (Bihar) and Rasalpur 12.26°N in Kodagu (Karnataka) and the results are presented in this paper.

MATERIALS AND METHODS

Cutworm larvae were collected from the field and reared in the laboratory. In the initial stages the larvae were reared in earthen pots covered with perforated polyethylene sheet and were later confined individually in glass jars closed with muslin cloth. Investigations on the parasitoids of *A. segetum* were conducted mainly at Simla. All the adult parasites which emerged were removed regularly and preserved in 70 per cent alcohol. Record was kept of all the cutworms reared and parasitoids emerged for calculating the percentage parasitism. The data are presented in Tables 1 and 2.

RESULT AND DISCUSSION

Greasy cutworm, Agrotis ipsilon *(Hufnagel)*: Though this species was recorded on potato from all the places observed, it was more abundant in Patna and Jullundhur forming 63.7 to 93.9 per cent of all the cutworms present. In March-April, thousands of moths were attracted to artificial light at Simla hills, but the actual damage caused by the caterpillars to potato was negligible. The caterpillars

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TABLE 1. Relative abundance (%) of cutworms on potato.

Place	Year of observation	Cutworm species (%)				
		Agrotis segetum	*A. ipsilon*	*A. spinifera*	*A. flammatra*	*Xestia c-nigrum*
Rasalpur (Karnataka)	1976	14.7	0.6	84.7	0.0	0 0
Simla (Himachal Pradesh)	1973	87.8	0.8	0.0	0.0	11.4
	1974	90.0	0.6	0.0	0.0	9.4
Jullundhur (Punjab)	1973	31.2	68.8	0.0	0.0	0.0
	1974	36.3	63.7	0.0	0.0	0.0
Patna (Bihar)	1968	0.0	93.9	2.3	3.8	0.0

were, however, observed feeding on succulent roots of wild host plants and ornamental plants in May. The moths appeared to migrate from the plains to the hills after emerging from the brood which fed on potato and/or gram. The moths were possibly helped by the seasonal winds in their to and fro migration between plains and hills. The non-migrating population of the noctuid was observed to cause serious damage to potato crop in March—April at Jullundhur. The late sown crops of potato were also severely attacked at Patna in February—March. The caterpillars fed on leaves, stems and tubers of the crop. The damage to the tubers alone was to the extent of 5 to 32 per cent at Patna.

Turnip moth, Agrotis segetum *(Denis & Schiff.)*: It was most abundant on potato in Simla hills (forming 87.8 to 90.0 per cent of total cutworm population) followed by Jullundhur (31.2 to 36.3 per cent) and Rasalpur (14.7 per cent). It hibernated during the winter months. In Simla hills, the tuber damage by this species ranged from 7 to 25 per cent.

Gram cutworm, Agrotis flammatra *Fabr.* This species was recorded feeding on potato only at Patna, its incidence was low forming 3.8 per cent of the cutworms. The moth was attracted to light and in March—April thousands of adults of *A. flammatra* along with *A. ipsilon* were attracted to artificial light in Simla. As in the case of *A. ipsilon* this noctuid also bred at Simla during hot months on wild hosts and ornamental plants.

Surface cutworm, Agrotis spinifera *(Hb.)*: This species was recorded as the most serious pest of potato in Rasalpur forming 84.7 per cent of the cutworm population and it also caused some damage to potato in Patna (2.3 per cent). In Rasalpur 37 per cent tubers were damaged by this cutworm. Adults of *A. spinifera* were attracted to artificial light in Punjab (Farid Kot, Ludhiana, Jullundhur) and Hyderabad but its absence on potato in these areas indicated that the pest preferred to feed on other hosts.

Spotted cutworm, Xestia c-nigrum *(L.)*: This noctuid was recorded on Simla hills causing damage to potatoes mainly by feeding on the leaves and stems and forming 9.4 to 11.4 per cent of the cutworms.

PARASITOIDS OF THE CUTWORMS:

Macrocentrus collaris (*Spin.*) (Figs. 1-2): This braconid was recorded as the most

Fig. 1. Cocoons of *Macrocentrus collaris*; 2. Adults of *M. collaris*; 3. Adult of *Netelia ocellaris* with its cocoon; 4. ♂*Coelichneumon* sp. nr. *truncatulus*; 5. ♀*Coelichneumon* sp. nr. *truncatulus*; 6. *Periscepsia carbonaria* with their puparia; 7. *Turanofonia chinensis*; 8. *T. chinensis* with its puparium; 9. Adult of *Enicosphilus* sp.; 10. *Neoaplectana* larva; 11. Pupa of *Agrotis segetum* with a ring of emerging larvae of *Neoaplectana* sp.; 12. Healthy caterpillar of *A. segetum*.

common parasitoid from *A. segetum* in Simla hills. The parasitoids hibernated as mature larvae mostly in the last instar caterpillar. Adult parasitoids started appearing in the 1st week of May, when the hibernating cutworm population started pupating. The female with its fairly long ovipositor deposited its eggs in the body of 2nd or 3rd instar caterpillars and the larvae when full fed came out and spun brownish cocoon in 2 to 3 days and the adults emerged in 14-17 days in May—June. The development of parasitoid was polyembryonic and as many as 70 larvae emerged from a single caterpillar. This parasitoid actually determined the fate of 1st generation of *A. segetum* on Simla hills. Parasitism was fairly high (20.87—42.5 per cent) and the low population of 1st generation of *A. segetum* in 1974 could be attributed to the effect of this parasitoid. This parasitoid had earlier been recorded on *A. segetum* in USSR (ALIEV, 1959; KAMENKOVA, 1967, 1968; ALEKCEEV, 1972) and from Czechaslovakia (BIROVA, 1973).

Netelia ocellaris *(Thomson) sp. complex*: (Fig. 3) This yellowish-brown ichneumonid was recorded as a solitary exoparasite from *A. segetum* on Simla hills. The parasitoid hibernated in its cocoon from which it emerged in the beginning of May. Parasitism of *A. segetum* caterpillars varied from 3.48 to 5 per cent. The full fed parasitoid came out of the body of final instar caterpillar and formed a dark brown (almost black) cocoon within 48 hours. The adult parasitoid emerged in 22—24 days.

Coelichneumon *sp. nr* truncatulus *Thomson*: (Figs. 4-5) This ichneumonid parasitoid was recorded for the first time from *A. segetum* on Simla hills and *A. ipsilon* in Jullundhur. The adult parasitoid exhibited

TABLE 2. Parasitoids reared from *Agrotis segetum* D & S in Simla.

Family/Species	Year/Percentage parasitism	
	1973	1974
Braconidae		
Macrocentrus collaris (Spin.)	20.9	42.5
Ichneumonidae		
Netelia ocellaris (Thomson) sp. complex	3.5	5.0
Coelichneumon sp. nr. *truncatulus* Thomson	10.4	11.2
Tachnidae		
Periscepsia carbonaria Panzer	4.3	7.5
Turanogonia chinensis Wiedemann	2.6	5.0

sexual dimorphism. The male had a yellow scutellum while in female it was dark brown. In most of the cases the female parasitoid emerged from the female pupa of the host and the male from the male pupa. The pupal weight of the male and female parasitized *A. segetum* pupae was on an average 165.71mg (range 145—210) and 235.71mg (145—350) respectively while that of healthy (unparasitized) *A. segetum* pupae was 255mg (180—380) and 301mg (180—390). The female parasitoid emerged from pupae in 16.43 days and the males in 14.14 days. The male and female moths of *A. segetum* emerged from the pupae in 15.3 days and 16.3 days. The parasitized *A. ipsilon* pupa weighed 200mg and unparasitized (healthy) 550mg. At Simla 10.43 to 11.25 per cent pupae of *A. segetum* were parasitized while at Jullundhur 8.3 per cent of *A. ipsilon* pupae were parasitized. This parasitoid also emerged from the pupae of *A. segetum* collected from Jullundhur. This parasitoid had earlier been recorded from pine processionary, *Thaumetopea pityocampa* (Schiff.) in Portugal (CARDOSO CARBRAL *et al.*, 1965).

Periscepsia carbonaria *Panzer*: (Fig. 6) This tachinid fly was reared from the caterpillars of *A. segetum* at Simla. From the caterpillars collected in the beginning of May and throughout June normally 3 maggots emerged from a single host, the puparia were formed within 4 days and the flies emerged in about 12 to 22 days. Although 4.35 to 7.5 per cent parasitism was observed in the caterpillars of *A. segetum* collected from the field and reared in the laboratory, yet the adult activity of the pest was very intense in the potato fields. They were seen moving very fast at low level near potato plants. The fly was also recorded parasitising this pest in Jullundhur.

Turanogonia chinensis *Wiedemann*: (Figs. 7—8) This parasitoid was recorded from *A. segetum* on Simla hills and at Jullundhur, and Rasalpur in Kodagu parasitising 2.61 to 5, 5, and 12.5 per cent caterpillars respectively. It was reared from *Xestia c-nigrum* in Simla, *A. ipsilon* in Jullundhur and *A. spinifera* at Rasalpur in Kodagu but the percentage parasitism never exceeded 5. It is a larvo-pupal parasite and only one parasite completed larval development in one host.

Apanteles ruficrus *Hal.*: This braconid was reared from *A. ipsilon* from Jullundhur and Patna parasitizing 6.8 and 9.6 per cent caterpillars respectively. With its white cocoons it could be easily distinguished in the field.

Enicosphilus *sp.*: (Fig. 9) This parasitoid emerged from the pupae of *A. segetum* and *A. spinifera* parasitizing 3.8 per cent of the former and 9.6 per cent of the latter at Rasalpur during 1976.

Some species of *Enicosphilus* such as *E. repentinus* Holmgr. and others were earlier recorded from *A. segetum* (YAKHONTOV, 1929; LUZETSKY, 1960).

Neoaplectana sp.: (Figs. 10—11) This entomophilic nematode was recorded parasitizing *A. ipsilon* and *A. segetum* in irrigated potato fields at Jullundhur and was found pathogenic to *Xestia c-nigrum* (L.) and white grubs at Simla (SINGH, 1977).

During 1976, 10.6 per cent of the caterpillars of *A. segetum* collected from Rasalpur were infected by this entomophilic nematode. *A. spinifera* was also severely infected.

The entomogenous fungus, *Metarrhizium anisoplae* (Metch.) Sorokin was recorded as commonly occurring in potato fields in Simla hills, where it infected cutworms, although it mainly multiplied on white grubs and wireworms (SINGH, 1978).

During March—April the common monkey, *Macaca mulatta* Zimmerman was observed sitting near street light and feeding on the adults of *A. ipsilon*, *A. flammatra* and other large sized noctuids. The monkey used to catch the moths, remove their wings and eat the remaining portions.

A sphecid digger wasp belonging to genus *Ammophila* was seen entering the hide outs of *A. segetum* caterpillars This digger wasp paralized the mature caterpillars and carried them to its nest one by one. This predator was most active in Simla although its exact role in suppression of *A. segetum* and other noctuids could not be determined.

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DESCRIPTION OF A NEW SPECIES OF *ONYCHOTRECHUS* KIRKALDY (HETEROPTERA:GERRIDAE) FROM INDIA

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(Received 16 January 1981)

Onychoterchus baijali, sp. nov., is described from Anamalai Hills, Tamilnadu, India.
(Key words: new species, Onychotrechus, Gerridae)

INTRODUCTION

Onychotrechus Kirkaldy is a small oriental genus, hitherto represented by two species from India, Burma and Ceylon. In the present contribution one new species is described, bringing the recorded fauna of the genus upto three species viz., *O. rhexenor* Kirkaldy, 1903, *O. sakuntala* (Kirkaldy), 1901 and *O. baijali* sp. n.

Onychotrechus baijali, sp. nov. (Figs. 1—10)

Size—Winged male 6.15 mm long; width across head including eyes 1.1 mm; width across humeri 1.35 mm; width of body across mesoacetabula 1.40 mm. Winged female 6.6 mm long; width across head including eyes 1.15 mm; width across humeri 1.42 mm; width of body across mesoacetabula 1.55 mm.

Colour—Upper surface yellowish brown with black markings. Head yellowish brown with a pair of black longitudinal stripes. Pronotum yellowish brown with a pair of black fuscous stripes on anterior lobe; posterior lobe black with yellowish brown margins between humeri. Propleuron with two black longitudinal stripes. Meso- and metapleural regions yellowish brown with a pair of black lateral longitudinal stripes. Abdominal tergum dark brown. Connexivum yellowish laterally. Antennae yellowish brown. Legs brownish. Hemelytra dark brown.

Structural Characteristics:

Head—Proportional length of antennal segments of winged male : 1st : 2nd : 3rd : 4th :: 26 : 20 : 20 . 23, total length of antenna 4.45 mm, of winged female: 1st : 2nd : 3rd : 4th :: 28 : 21 : 21 : 24, total length of antenna 4.7 mm. Head including eyes wider than long (22:17 in male and 23:18 in female). Eyes with inner margin emarginate. Antenniferous tubercles poorly defined. Clypeus with distinct basal margin. Mandibular and maxillary plates distinct from each other. Rostrum slender, long, extending anterior one third of mesosternum, third segment three and a half times as long as lastsegment (28 : 8 in male and 30 : 8.5 in female).

Thorax—Pronotum a little less wide then head including eyes, posterior margin broadly rounded. Intersegmental suture between mesonotum and metanotum clearly defined dorsally as well as laterally. Mesosternum about five times as long as metasternum (36:7 in male and 40:8 in female); median longitudinal sulcus well marked in anterior half only; paired longitudinal sutures distinctly present anteriorly. Metacetabular suture obscure, not reaching lateral longitudinal suture

The relative length of leg segments.

	Femur	Tibia	First tarsal segment	Second tarsal segment	Total tarsal length
Winged male (6.15 mm)					
Fore leg	41	36	2.5	6.2	8.7
Mid leg	118	86	4	8	12
Hind leg	132	82	4.5	9	13.5
Winged female (6.6 mm)					
Fore leg	42	36	2.6	6.5	9.1
Mid leg	122	88	4.5	9	13.5
Hind leg	138	84	4.8	9.8	14.6

of metanotum dorsally. Metasternum about twice as long as second ventrite (7:3.5 in male and 8:4 in female). Omphalium distinct, located at about posterior one third of metasternum. Omphalial groove absent. Fore leg slender; femur thicker and a little longer than tibia, with two spines on the inner surface at the distal one fourth of male; tibia slightly swollen apically; second tarsal segment about two and a half times as long as first segment, claws arising from apical one third of second segment.

Wing venation—Hemelytra with *Sc* connected to *RM* at the point of bifurcation into *R* and *M* by an oblique vein Sc_2.

Abdomen—Abdomen elongate, strongly narrow posteriorly. Connexivum without spine. Abdominal segments unequal in length and shape. Abdominal ventrites increasing longer posteriorly; with a median longitudinal depression in male. Abdominal spiracles located at middle of each segment.

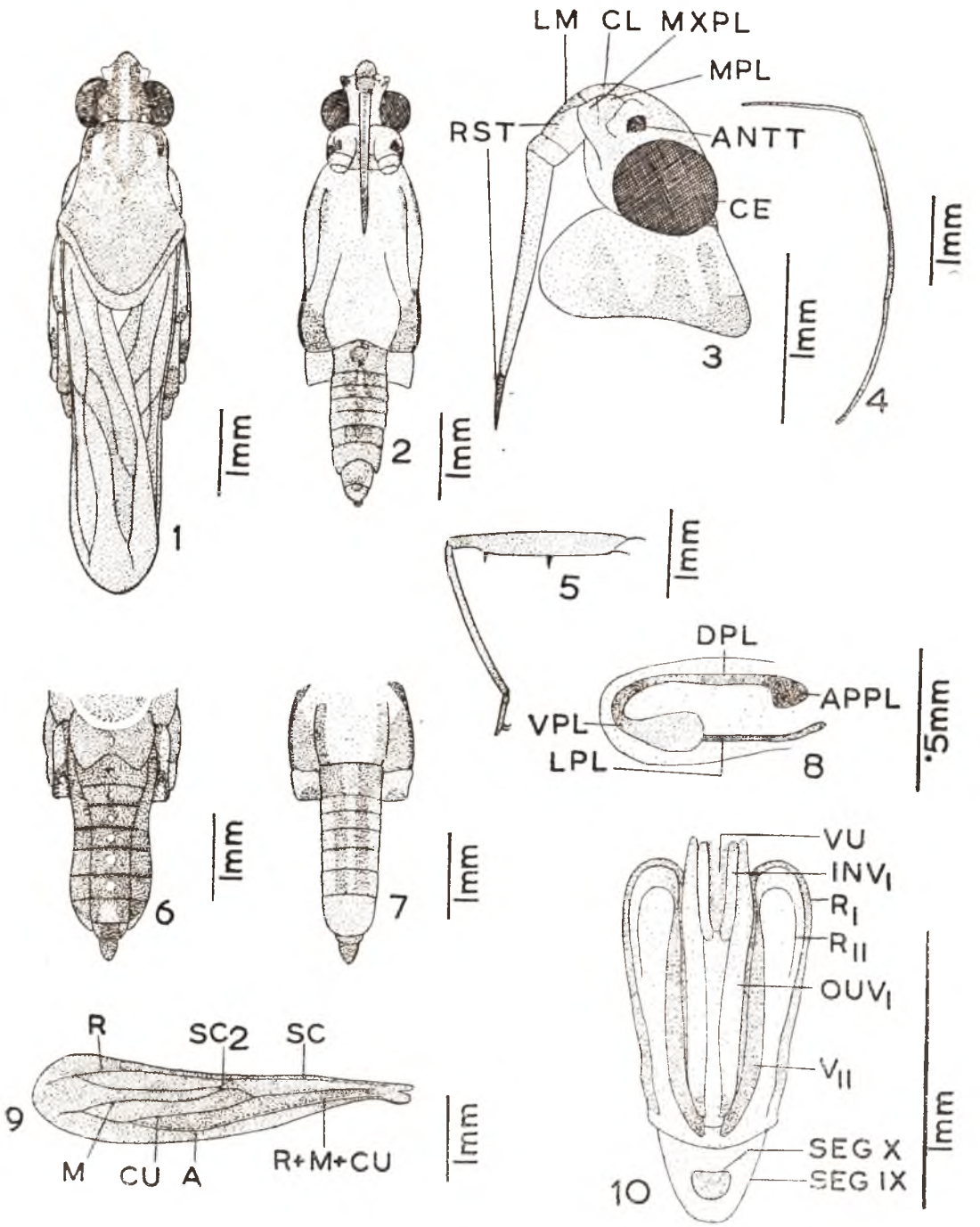
Male genitalia—Seventh segment concave on ventral posterior margin, without connexival spine. Eighth segment well developed with apical margin concave. Ninth segment with suranal plate simple; pygophore clearly visible ventrally; parameres short. Endosoma with dorsal plate fused with round apical plate on apical margin; lateral plate slender; ventral plate completely membranous, without sclerotized basal support.

Female genitalia—Seventh segment well developed dorsally as well as ventrally. Eighth segment ventrally not fully visible; first valvula with inner lobe narrow, short, about one third as long as outer lobe; outer lobe simply narrow apically, pointed at tip. Second valvula narrow posteriorly, sharp at apex, extending beyond apical margin of intervalvular membrane. Vulva totally membranous, connected with inner lobe of first valvulae.

Material examined — **Holotype** one winged male; **allotype** one winged female on pins; **paratypes** winged two males, three females. TAMIL NADU, Anamalai Hills, Cinchona, 3500 ft., 28.v.1962 (P. Susai Nathan). Types at present with the author and will be deposited in the National Collection with the Z. S. I., Calcutta.

Distribution—INDIA: Tamil Nadu.

Remarks—This species comes close to *O. sakuntala* (Kirkaldy). It can readily



Onychotrechus baijali, sp, nov. Fig. 1—Dorsal view of winged male; 2—Ventral view of winged male (Wings removed); 3—Lateral view of head; 4—Antenna of male; 5—Fore leg of male; 6—Dorsal view of female abdomen (Wings removed); 7—Ventral view of female abdomen (Wings removed); 8—Apical segment of endosoma; 9—Hemelytra; 10—Female genitalia.

APPL Apical plate; ANTT, Antenniferous tubercle; CE, Compound eye; CL, Clypeus; DPL, Dorsal plate; LM, Labrum; LPL, Lateral plate; MPL, Mandibular plate; MXPL, Maxillary plate; R, Ramus; RST, Rostrum; SEG, Segment; V, Valvula; VF, Valvifer; VPL, Ventral plate; VU, Vulva; INV_1, Inner lobe of first valvula; OUV_1, Outer lobe of first valvula.

be defferentiated by the presence of two spines on the inner side of male fore femur, second tarsal segment twice as long as first tarsal segment in mid leg, third segment of rostrum three and a half times as long as fourth segment.

This species is named in honour of Dr. H. N. Baijal in recognition of his outstanding contribution in the field of Insect Taxonomy as well as for his generous assistance to the author.

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AN UNDESCRIBED SPECIES OF *AMEMBOA* ESAKI (HETEROPTERA : GERRIDAE) FROM INIDA

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A new species of *Amemboa* Esaki, A. *keira* is described from India : West Bengal and Sikkim
(Key words: Amemboa species, Heteroptera, Gerridae)

Esaki (1925) erected the genus *Amemboa* with the type-species *Amemboa fumi*. *Amemboa* is an oriental genus, hitherto comprised of seven species from India, Burma, Malaya, Java, Sumatra, Philippines, Formosa, Thailand and Vietenam, only two of which *A. kumari* (Distant), 1910 and *A. pervati* Pradhan, 1951 were recorded from India. In the present paper one new species is described, bringing the recorded fauna of India upto three species.

Amemboa keira sp. nov. (Figs. 1—9).

Size—Apterous male 3.6 mm long; width across head including eyes 0.67 mm; greatest width of body across mesoacetabula 1.5 mm. Apterous female 4.15 mm long; width across head including types, 0.77 mm; greatest width of body across mesoacetabula 1.77 mm.

Colour—Upper surface predominantly yellowish with reddish brown markings. Venter pale yellow. Antennae and legs light yellow.

Structural characteristics:

Head—Proportional length of antennal segments of apterous male: 1st: 2nd: 3rd: 4th :: 14: 12: 13: 24, total length of antenna 3.15 mm, of apterous female: 1st: 2nd: 3rd: 4th :: 15: 13: 14: 25, total length of antenna 3.35 mm. Head including eyes nearly as wide as long (13.5: 13.2 in male and 16.5: 16.3 in female). Antenniferous tubercles indistinct. Rostrum slender, a little surpassing posterior margin of prosternum.

Thorax—Pronotum distinctly shorter than head, wider than head including eyes. Intersegmental suture between mesonotum and metanotum obscure laterally. Mesosternum about ten times as long as metasternum; median longitudinal sulcus indistinct; paired longitudinal sutures absent. Metacetabular suture not extending lateral elevation of metanotum. Metasternum about three times as long as second ventrite. Omphalium located a little more towards the posterior margin of metasternum. Fore leg exihibits sexual dimorphism. Male fore femur relatively thicker and with comb of closely set short, dark brown hairs on basal and apical inner ends.

Abdomen—Abdomen elongate, subtriangular in shape. Connexivum without spine. Abdominal spiracles hidden beneath greatly developed meso- and metacetabula, located at about middle of each segment. Ventral median longitudinal carina lost.

Male genitalia—Seventh ventrite simply concave apically. Eighth segment cylindrical, ensheathing ninth segment basally,

The relative length of leg segments.

	Femur	Tibia	First tarsal segment	Second tarsal segment	Total tarsal length
Apterous male (3.6 mm)					
Fore leg	21	18	3	5.5	8.5
Mid leg	55	39	15	8	23
Hind leg	52	22	10	7	17
Apterous female (4.15 mm)					
Fore leg	23	19	3	5.8	8.8
Mid leg	59	41	16	8.1	24.1
Hind leg	56	23.5	10.5	7	17.5

ventrally a little longer than combined length of the entire preceding abdominal segments. Ninth segment with suranal plate differentiated into three regions. Pygophore broadly rounded on apical margin; parameres not developed. Endosoma elongated, connected with apical plate apically; apical plate paired, rounded on apical margin; ventral lobe small, totally membraneous; lateral plate slender, elongated.

Female genitalia — Seventh segment well developed. Eighth segment exposed only dorsally, completely covered by seventh segment ventrally; first valvula with inner lobe acute at apex, reaching a little less than half way of outer lobe; outer lobe narrow apically, sclerotized on inner half. Ninth segment with second valvulae sclerotized along lateral and apical margins, rounded apically, extending a little beyond apical margin of intervalvular membrane. Vulva membraneous.

Material examined—**Holotype** one apterous male; **allotype** one apterous female on pins; **paratypes** apterous 2 males, 1 female, West Bengal, Darjiling, Kalimpong, 16. x. 1978 (Y. C. Gupta); *other material examined:* Sikkim, Gangtok, apterous 4 males, 1 female, 21 x. 1978 (Y. C. Gupta). The types with the author and subsequently would be deposited with F R. I. Dehra Dun.

Distribution INDIA: West Bengal & Sikkim

Remarks—The specimens of *Amemboa keira* sp. nov. resemble those of *Amemboa lyra* (Paiva) but can be easily distinguished as the male anterior femur of *Amemboa lyra* (Paiva) is provided with a comb of short, uniform, closely set brownish hairs on the entire inner side but in *Amemboa keira* sp. nov. the male anterior femur is provided with comb of short, uniform, closely set brownish hairs only at the basal and apical ends on the inner side.

Acknowledgements:—I am thankful to Dr. H. N. Baijal, Zoology Department, Agra College, Agra, and to Dr. G. K. Srivastava, Supdt. Zoologist, Zoological Survey of India, Calcutta for going through the manuscript critically and affording valuable suggestions and to the University Grants Commission, New Delhi for financial assistance.

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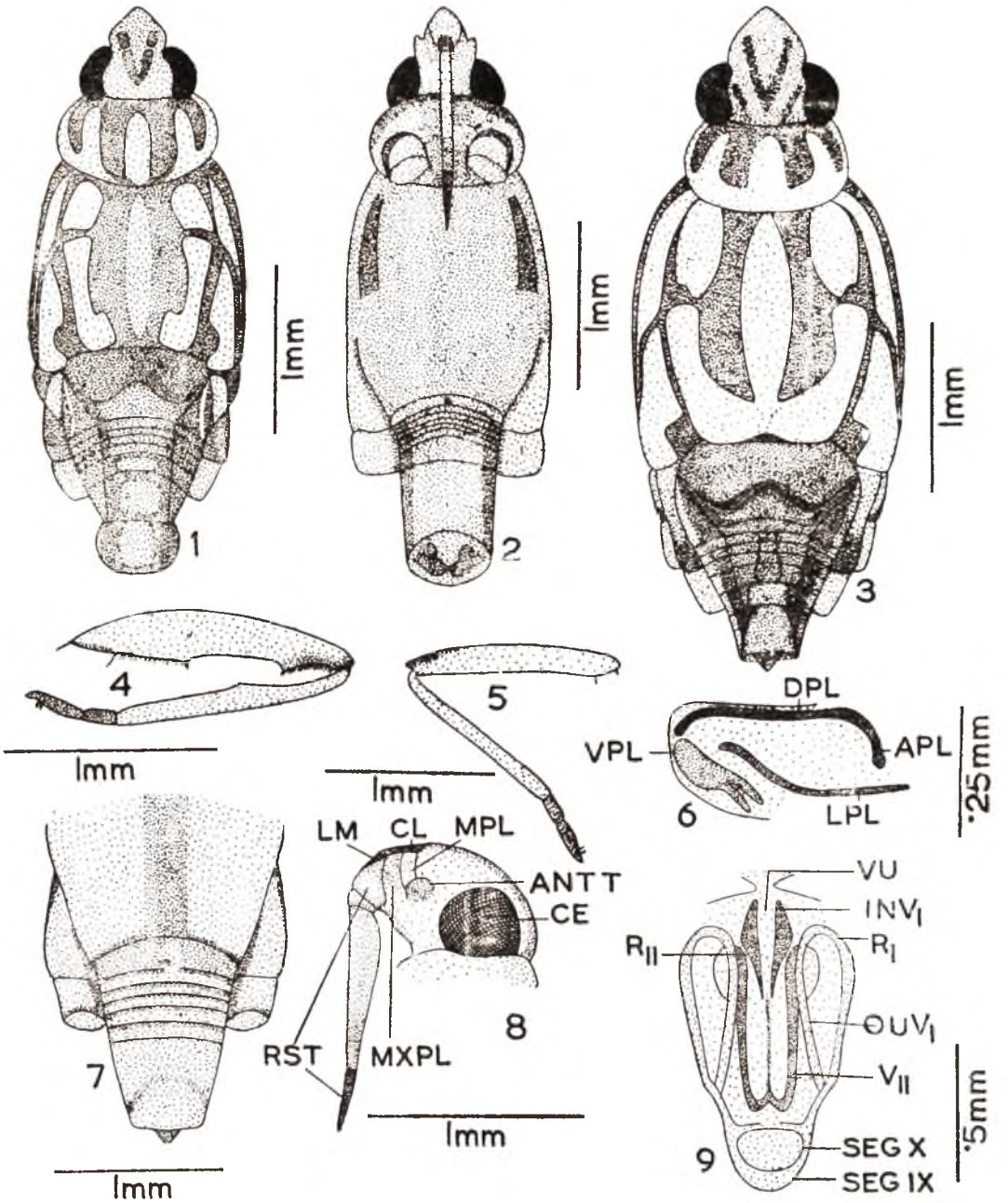

Amemboa keira., sp. nov. (Figs. 1—9): 1—Dorsal view of apterous male; 2—Ventral view of apterous male; 3—Dorsal view of apterous female; 4—Fore leg of male; 5—Fore leg of female; 6—Apical segment of endosoma; 7—Ventral view of female abdomen; 8—Lateral view of head; 9—Female genitalia.

APL, Apical plate, ANTT, Antenniferous tubercle; CE, Compound eye; CL, Clypeus; DPL, Dorsal plate; INV_1, Inner lobe of first valvula; LM, Labrum; LPL, Lateral plate; MPL, Mandibular plate; MXPL, Maxillary plate; OUV_1, Outer lobe of first valvula; R, Ramus; RST, Rostrum; SEG, Segment V_{11}, Second valvula; VU, Vulva.

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BRIEF COMMUNICATION

A TECHNIQUE FOR LABORATORY MULTIPLICATION OF *BRACHYMERIA NOSATOI* HABU AND OTHER SPECIES OF CHALCIDID PARASITOIDS OF *NEPHANTIS SERINOPA* MEYRICK[1]

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(Received 11 April 1981)

A technique for mass culturing the chalcidid *Brachymeria nosatoi* Habu, a solitary pupal parasitoid of the coconut caterpillar *Nephantis serinopa* Meyr. was standardised. It parasitised only host pupae remaining inside cocoons in silken galleries. The other species of *Brachymeria* such as *B. nephantidis, B. hime attevae* and *B. lasus* could also be reared in the laboratory using the same technique. *Brachymeria* spp. generally accepted fresh pupae as well as those which were in the advanced stage of development.

(Key words: breeding technique : *Brachymeria nosatoi : Nephantis serinopa)*

For several years now many parasite breeding laboratories in our country have been multiplying and releasing the gregarious larval parasitoids such as *Perisierola nephantidis* MUES. and *Bracon brevicornis* WESM., and the pupal parasitoid *Trichospilus pupivora* FERR. for the biological suppression of the coconut caterpillar, *Nephantis serinopa* MEYRICK. One of the primary considerations for choosing these parasites was that they were easily amenable to laboratory rearing and sizeable numbers of them could be multiplied using simpler techniques. NAGARKATTI (1973) observed that one obvious defect in the parasite rearing programme for the biocontrol of *N. serinopa* was that only those parasites which could be easily bred in the laboratory had been mass bred and released, while little had apparently been done with those parasites that were difficult to breed.

In addition to *Brachymeria nephantidis* GAHAN reported by earlier workers, five more species of *Brachymeria* have been recorded during 1972—1973, viz. *B. nosatoi* HABU (JOY & JOSEPH, 1972), *B. lasus* (WALKER), *B. excarinata* GAHAN (JOY *et al.*, 1973), *B. hime attevae*, JOSEPH *et al.* and *B. euploeae* (WESTW.) (JOY & JOSEPH, 1973), as primary pupal parasitoids of *N. serinopa*. Among these, *B. nosatoi* is the dominant species followed by *B. nephantidis*. The remaining species play a relatively insignificant role in suppressing *Nephantis* populations. According to JOY & JOSEPH (1977) parasitisation by *B. nosatoi* ranged from 0—28.7% with a mean of 9.9%. The aggregate parasitism by *B. nosatoi* in southern Kerala was 19.3%, whereas in northern Kerala it was only 2.2%. Our studies during the past three

[1] Contribution No. 557 of CPCRI, RS, Kayangulam.

years (1978—1980) also revealed higher intensity of natural parasitism of *N. serinopa* pupae by *B. nosatoi* and *B. nephantidis* in the southern districts of Kerala. Out of an aggregate pupal parasitism of 41.6% by *Brachymeria* spp. (1080/2596 pupae), *B. nosatoi* had effected 24.26%, *B. nephantidis* 16.45%, *B. hime attevae* 0.5% and *B. lasus* 0.38% parasitism. It was therefore decided to standardise a suitable technique for rearing *B. nosatoi,* which though a solitary parasite, has got great potential in suppressing *N. serinopa.* This parasite was hitherto considered to be not amenable to laboratory rearing. We could successfully rear it in the laboratory by simulating field conditions. It is now possible to mass culture this parasite and augment its population in nature. Details of the rearing technique which we have developed are discussed in this short communication.

A nucleus culture of *B. nosatoi* for laboratory multiplication was obtained from field collected *N. serinopa* pupae kept under observation for parasite emergence. The pupae parasitised by *Brachymeria* spp. show one or more black dots, which are the characteristic oviposition punctures made by the females. Thirty to fifty adults of *B. nosatoi* comprising both sexes are sorted out and released into a cylindrical glass jar, 17.5 cm long and 6.75 cm wide. The mouth of the jar is covered with muslin cloth and held by rubber bands. Honey is provided as food for parasites as small droplets on a piece of wax coated paper or butter paper. The jar containing parasites is kept in subdued sunlight for 10—15 minutes daily for about 3—4 days, after which only the host pupae are offered for oviposition. Exposure to sunlight stimulates mating. Pupae of *N. serinopa* reared in the laboratory are carefully removed with cocoons and silken galleries intact or leaf bits bearing pupae within cocoons and galleries are placed on a piece of cardboard 12 cm long and 6.25 cm wide. The cardboard piece with several pupae is inserted into the horizontally placed glass jar containing mated parasites for oviposition (Fig. 1).

The parasites readily oviposit in the pupae standing on the galleries and partially disorganising the pupal tissues with their ovipositors by repeated thrusts. Those pupae without cocoons and silken galleries are to be placed on the cardboard and covered with silken galleries. Superparasitism generally occurs when the pupae are exposed for parasitisation for longer periods. As many as 23 eggs were observed to be laid in a single pupa which was subject to heavy superparasitism. If supernumerary eggs are laid in a single host pupa, the hatching parasite larvae are likely to compete for nutrition. In such cases generally none of them survives and as such, no adult parasite emerges from heavily superparasitised pupae. To avoid superparasitism the parasitised host pupae are to be removed immediately after oviposition by parasites. Depending on the activity of female parasites, the host pupae can be exposed for a period of 4—6 hours for parasitisation. Then the cardboard piece containing parasitised pupae has to be transferred to a similar glass jar or the parasitised pupae alone to a conical flask and kept for emergence. Parasite emergence normally commences 12 days after oviposition and continues up to 20 days in the laboratory at temperature and R. H. ranging between 22—30°C and 45—80% respectively.

For rearing other species of chalcidids such as *B. nephantidis, B. hime attevae* and *B. lasus* naked pupae or pupae within the cocoons and silken galleries can be exposed on a piece of cardboard. All

Fig. 1. Cage for breeding *Brachymeria nosatoi* HABU.

of them readily oviposit in naked pupae. If pupae within cocoons and silken galleries are offered, the female parasites bite and make small holes in the cocoons and through these oviposit in the pupae. *B. lasus* generally prefers large host pupae. Small host pupae produce a higher proportion of males. *Anadevidia (= Plusia) peponis* pupae are suitable for rearing *B. lasus* successfully. Pupae of *Herculia nigrivitta* WALKER infesting dried coconut leaves, can also be used for rearing *B. nephantidis* and *B. hime attevae*. This study showed that the *Brachymeria* spp. accepted hosts of any age.

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BRIEF COMMUNICATION

THE POSSIBLE ROLE OF RESERVOIR PLANTS IN THE MAINTENANCE OF THE BRINJAL PEST *HENOSEPILACHNA (EPILACHNA) VIGINTIOCTOPUNCTATA* FABR (COLEOPTERA: COCCINELLIDAE) THROUGHOUT THE YEAR

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Attack of brinjal crop by *H. vigintioctopunctata* occurs throughout the year in Anuppanadi area of Madurai. Insecticide application gives only temporary protection. When insecticide is applied to brinjal, other solanceous plants offer shelter to the pest. Laboratory studies on the fecundity, duration of life cycle and survival percentage on different host plants support this view. It is suggested that for pest eradication control measures must be extended to the other solanaceous plants also.

(Key words: reservoir plants, fecundity, survival percentage)

Henosepilachna (Epilachna) vigintioctopunctata FABR. is a common pest attacking cultivated solanaceous vegetables (DAVID, 1978) and medicinal plants (MATHUR & SRIVASTAVA, 1964). All the different developmental stages of *H. vigintioctopunctata* occur throughout the year on brinjal *Solanum melongena*, in Anuppanadi sewages fed farms near Thiagarajar College, Madurai. Periodic field observations (once in a fortnight) revealed that whenever insecticide is applied to brinjal crop, the pests become scarce on the crop but could invariably be found on other solanaceous plants like *S. nigrum*, *S. torvum* and *Lycopersicum esculentum* growing wildly among Amaranthaceae crop and from *Physalia minima* and *Datura fastuosa* growing near the hedges. This suggested that these plants offer shelter to the pest and maintain its population throughout the year. The present study tries to assess the possibility of these non-brinjal solanaceous plants acting as pest reservoir on the basis of fecundity, duration of life cycle and the percentage of survival of *H. vigintioctopunctata* on the plants.

The leaves of solanaceous plants *S. nigrum*, *S. torvum*, *L. esculentum*, *P. minima* and *D. fastuosa* grown in the College green house and protected from insecticides alone were used for rearing *H. vigintioctopunctata*.

H. vigintioctopunctata collected from the field were reared on brinjal leaves in the laboratory at room temperature in insect cages. The egg masses from this stock culture were used for further investigations. The first instar larvae were transferred on to the leaves of the experimental plants, using camel hair brush. Each experimental culture of *H. vigintioctopunctata* on the six different host plants was fed *ad libitum* with fresh leaves on alternate days for six consecutive generations.

Results presented (Table 1) showed that the fecundity of *H. vigintioctopunctata*

TABLE 1. The fecundity, duration of life cycle and percentage survival of *H. vigintioctopunctata* on various solanaceous host plants.

Host plants	Fecundity no. of eggs laid per day	Duration of life cycle no. of days from egg laying till adult emergence	Survival percentage of adults emerging per egg batch
Solanum melongena	30.15± 8.07	21.92±0.75	16.65±1.45
Solanum nigrum	62.25±15.64	23.86±0.37	14.80±3.41
Solanum torvum	3.20± 2.17	24.60±0.22	13.52±2.22
Lycopersicum esculentum	57.10±11.85	21.43±0.63	3.92±0.91
Physalis minima	34.90± 6.89	16.64±0.68	23.88±1.85
Datura fastuosa	25.85± 7.29	25.22±0.49	5.51±0.91

varies when reared on different host plants *S. nigrum* and *L. esculentum* to a remarkable extent and *P. minima* to a lesser extent promote a higher rate of egg laying than brinjal. The rate of egg laying is lower on *D. festuosa* and *S. torvum*. The duration of life cycle from egg laying till adult emergence is similar when reared on brinjal and *L. esculentum*. On *S. nigrum*, *S. torvum*, and *D. fastuosa* the duration of life cycle is progressively longer and on *P. minima* it is very much shortened. When compared to brinjal, the percentage of adults surviving from each egg mass is higher only on *P. minima* and it is progressively lesser on *S. nigrum*, *S. torvum*, *D. fastuosa* and *L. esculentum*.

On the basis of fecundity, duration of life cycle and survival percentage the highest potential for maintaining this pest is shown by the wild plant *P. minima* on which the pest shows the highest survival, shortest life cycle duration and a high fecundity rate. *S. nigrum* can act as a good alternative host because the fecundity rate is two times higher than on brinjal, even though the duration of life cycle is slightly longer and survival percentage is lower than on the latter. On *L. esculentum* the lower survival percentage of the pest is compensated by a higher fecundity rate and a duration of life cycle similar to brinjal. The data suggests that these three plants, viz. *P. minima*, *S. nigrum* and *L. esculentum* can maintain the pest population as effectively as brinjal. The other two solanaceous plants examined in the present study *D. fastuosa* and *S. torvum* are less preferred by the pest. On these two plants, fecundity is lower, duration of life cycle is longer and survival rate is much lower for the pest to occur on them when other host plants are available in the fields. However, they might offer shelter to the pest in the absence of suitable host plants as was confirmed by field studies. The present study strongly suggests that other naturally occurring solanaceous plants in the fields can maintain the population of this brinjal pest when the latter are temporarily protected by insecticide application.

Acknowledgement: We express our sincere thanks to the college authorities for the facilities provided.

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BRIEF COMMUNICATION

SOME OBSERVATIONS ON THE INSECT PESTS OF CINNAMON IN SRI LANKA

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The major pests affecting cinnamon *(Cinnamomum zeylanicum* Blume) in southern coastal belt of Sri Lanka are *Trioza cinnamomi* Boselli (Triozidae), *Chilasa clytia* Lankeswara and *Graphium sarpedon* Fd (Papilionidae) and *Orthaga vitialis* Walk. (Pyralidae). The minor pests are *Euproctis fraterna* Moore, and *Dasychira mendosa* Hou (Lymantriidae), *Thalassodes* spp. (Geometridae) and *Eudemopsis archemidias* Meyr. (Torticidae).

(Key words: insect pests, of cinnamon, *Cinnamomum zeylanicum*, Sri Lanka)

Sri Lanka is the major producer of cinnamon (*Cinnamomum zeylanicum* Blume) in the world from very early times (WIJESEKERE, 1975), and contributes about 60% of the world export market of this spice. The plant is subject to attack by a variety of insect pests during its different stages of development, causing considerable reduction in the yield of cinnamon bark and the quality of other products such as cinnamon leaf oil, bark oil and root bark oil. AYYAR (1940) reported nymphs and adults of *Pauropsylla depressa* C. as producing galls on leaves and shoots of cinnamon in India. MANI (1973) recorded 5 species of insects and mites producing galls on leaves and an unknown insect causing galls on inflorescence. The present paper embodies results of studies undertaken in Sri Lanka for the first time on the pests affecting the crop. These observations were made at routine visits to cinnamon plantations in Matara district and the experiments on biology was conducted at the Department of Agronomy, Ruhuna University, Matara since 1979 January. Field observations were performed by studying the various pests in cinnamon plantations in Matara district.

Trioza cinnamomi Boselli (Homoptera : Triozidae): This jumping plant louse is associated with the foliage of *C. zeylanicum*. The eggs are laid singly or in clusters on the upper or lower surface on newly emerged tender leaves. Feeding of the nymphs stimulates development of pale green to yellowish galls on the leaf surface. This is the most serious pest of cinnamon in Sri Lanka. The galls are mostly epiphyllous and sometimes also hypophyllous, ovoid or conical and unilocular and are located on the leaf blade and on the terminal buds. There may be numerous generations during the year, their durations approximately 35—45 days vary according to the season. After emergence of the adult the gall dries and turns dark brown in colour. Formation of galls results in major crop loss.

Chilasa clytia Lankeswara (Lepidoptera: Papilionidae): This butterfly is widely distributed in the cinnamon growing tracts of Sri Lanka. The butterfly is abundant from December to June and lays eggs singly on the upper and lower surfaces of young leaves, petioles and even on tender shoots. Eggs are small, round and pale yellow in

colour. The incubation period lasts for 3—5 days. The larval stage, comprising 5 instars, is completed in 12—18 days. Newly hatched caterpillar is more or less cylindrical, slightly green with one pale yellow dorsal line and irregular whitish stripes. When full grown the caterpillar is pale yellow with dark stripes on the sides of the body. The prepupal stage lasts for one day. It becomes sluggish before pupation and makes a rough silken padding on the stem and attaches itself to it by its anal end and remains attached to the stem by two silken girdles. The pupal period lasts for 11—14 days. The total life cycle lasts for 25—37 days.

The first instar caterpillar starts feeding on the lamina of the freshly emerged leaves while the subsequent instars feed on the tender leaves voraciously leaving only the mid ribs with portions of veins.

Graphium sarpedon Fd. (Lepidoptera: Papilionidae): This butterfly is common, all the year round in cinnamon growing areas. The eggs are laid on the underside of the tender leaves. The egg is round smooth and yellow in colour. The egg period lasts for 5—6 days. The first instar caterpillar is spiny and smoky in colour. They first keep to the underside of the leaves feeding on them and later seem to favour the mid rib on the upper side of the leaves. As they grow they become green, the larval stage is comprised of 5 instars occupying 29—31 days. They pupate on the underside of the leaves and pupal period lasts for 19—20 days. The period from oviposition to emergence of adult is about 59—60 days.

G. doson Fd. is also observed to feed on tender cinnamon leaves in Sri Lanka.

Orthaga vitialis Walk. (Lepidoptora: Pyralidae): The caterpillar of this moth webbs up the leaves into clusters. The leaves webbed up gradually dry up and are held together by the web. Eggs are laid in small clusters on the silken webbing or singly on ribs of leaves by the female moth. The incubation period lasts for 4—5 days. The larvae are gregarious in the beginning and feed by scraping the leaf surface. They soon make tunnels of thin webs, within which they remain feeding. The larvae moult 5—6 times and become full grown in about 28—30 days. The fully fed larva is pale green with dark bands. A webbed up cluster of leaves harbours several larvae. Pupation takes place within a silken cocoon in the webbed up cluster for 11—14 days. Adult life lasts for 4—5 days in the laboratory. A severely infested plant shows many such clusters on it and presents a sickly appearance.

Euproctis fraterna Moore (Lepidoptera : Lymantriidae): Feeding of this larva on the leaves is mostly seen during August — December. The eggs are laid in groups on the leaves and are covered with hairs from the body of the female. The egg period lasts for 4—9 days. The larval period lasts for 13—29 days including 6 larval instars. When the larvae are fully grown they feed gregariously on leaves. It pupates in a silken or hairy cocoon. The pupal period lasts for 9—20 days. The length of the life cycle is about 6—7 weeks. The larva is active during the flushing season and causing extensive damage.

E. irrotata Moore is also seen destroying the tender leaves, in Sri Lanka.

Dasychira mendosa Hbn. (Lepidoptera : Lymantriidae) : The larvae of this moth feed on foliage of cinnamon. The female moth lays masses of yellow eggs on underside of leaves. The egg period lasts for 3—4 days. Larvae feed on the leaves, the larval period lasting for 21—28 days. They pupate in loose cocoons made of silk and pupal period lasts for about 11—12 days.

Thalassodes spp. (Lepidoptora: Geometrida): This looper caterpillar causes damage by feeding on the tender leaves of cinnamon. Eggs are laid in small groups or singly on edges of the tender leaf blades. Eggs hatch in 2—3 days. The larval period lasts for 17—18 days. The larva possessing the colour of the new shoot and assuming a characteristic pose on the twing is often mistaken for a leaf petiole. The pupal period lasts for 7—8 days and the pupa is of the colour of tender leaves later turning to brown colour. The total cycle is completed in 26—29 days.

Phyllocnistis citrella Statinton (Lepidoptera: Phyllocnistidae): The adult is a tiny, silvery moth. The female moth lays minute flat eggs singly on the lower surface of the leaves close to the midrib and hatch in about 2—6 days. The greenish yellow young caterpillar enters the leaf tissue by mining. The fully grown caterpillar turns red. The pupal period lasts for 6—22 days in different seasons. The mined leaves turn pale and curl up and the development of young leaves is retarded.

Olethreutes semiculta Meyr. (Lepidoptera: Tortricidae): The larva rolls together the young leaves. Egg period lasts for 2—8 days. There are 5 larval instars completed in 12—53 days depending upon the climate. Pupation takes place in silken cocoon beneath the folded edge of a leaf for 7—24 days. The total life cycle takes 21—85 days for completion.

Zeuzera coffeae Nietu. (Lepidoptera: Zeuzeridae): The red caterpillar bores into the stem and branches and causes minor damage. The moth lays a string of yellow eggs in cracks and crevices on the stems and branches. Eggs hatch in 7—8 days. The larva bores into the stem feeding and tunnelling within it. It pupates within the larval tunnel for 3—7 weeks. The smaller branches if attacked dry up soon and the attack is confined mostly to the base of the tree.

Other insect pests observed in cinnamon plants are *Attacus atlas* L. (Lepidoptera: Saturniidae), *Argyroploce aprobola* Meyr. (Lepidoptera: Eucosmidae), *Eudemopsis archemidias* Meyr. (Lepidoptera: Tortricidae), *Coptosoma pygmaeum* Mont. (Heteroptera: Plataspidae), *Leptocentrus obliquus* Walker (Homoptera: Membracidae), *Coenobius lateralis* Weise (Coleoptera: Chrysomelidae), *Cryptocephalus snillus* Suffr. and *C. virgula* Suffr. (Coleoptera: Chrysomelidae), *Podagrica badia* Harold (Coleoptera: Chrysomelidae), *Centrocorynus dohrni* Jekwl (Coleoptera: Attebalidae), *Microspathe fuliginosa* Pascoe (Coleoptera: Curculionidae), *Evorinea hirtella* Walker (Coleoptera: Dermestidae) and *Eriophyes* spp. (Acarina: Eriophyidae).

Oecophylla smaragdina F. (Hymenoptera Formicidae) causes nuisance due to the formation of nests on leaves. The leaf scale *Ceroplastes rubens* Mask (Homoptera: Coccidae) is also observed to infest the leaves, causing the leaves to turn black due to sooty mould growth.

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BRIEF COMMUNICATION

A NOTE ON *TRICHOSPILUS PUPIVORA* FERR., A PARASITE OF *NEPHANTIS SERINOPA* MEYR.[1]

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(Received 11 April 1981)

Part of the population of the pupal parasite *Trichospilus pupivora* Ferr. (Eulophidae) was observed to aestivate in the pupal stage during March-May in the field and laboratory. Low searching and dispersal ability, low tolerance for non-optimal temperatures, poor sense of discrimination between parasitised and unparasitised host which leads to superparasitism and inability to compete with other pupal parasites such as *Brachymeria* spp. are the major factors that contribute to the inefficiency of the parasite and its lower intensity in the field.
(Key words: aestivation, efficacy, extent of pest suppression, *Trichospilus pupivora* Ferr.)

The gregarious pupal parasite *Trichospilus pupivora* FERR. (Hymenoptera: Eulophidae) is being mass multiplied and used as a biotic agent for suppressing the population of the leaf eating caterpillar *Nephantis serinopa* MEYRICK on coconut in India and Sri Lanka since many years. This parasite cannot tolerate high temperature and low humidity conditions during summer months, and as such, its multiplication in the laboratory during March—May period is a major bottleneck. Natural parasitism by *T. pupivora* reaches negligible levels during the summer season. ANANTHANARAYANAN (1934) also reported that *T. pupivora* is greatly influenced by weather changes.

We have observed that a part of the population of *T. pupivora* aestivates in the pupal stage during March—May in the field as well as in the laboratory to tide over the unfavourable weather conditions. Aestivation in the field commences from early March, when the minimum and maximum temperatures range between 22.2—24.2°C and 34—35.9°C respectively and R. H. 51—94%. Normally the egg to adult stages of *T. pupivora* are completed in 14—16 days (egg period 2 days, larval period 5—6 days and pupal 7—8 days) under the west coast conditions, whereas the aestivating pupae remain quiescent for about 4—6 weeks, as against the normal pupal duration of one week. In the laboratory culture also, part of the population undergoes aestivation from early March onwards. Emergence of adult parasites from laboratory cultures of parasitised pupae is quite erratic, commencing 22, 24 and 31 days after oviposition, as against the normal developmental period of 14—16 days. Adult parasites which emerge during the summer season are comparatively weak, inactive and undersized and generally die in about 1—4 days.

Although *T. pupivora* is a gregarious parasite, it fails to check population of *N. serinopa* in coconut gardens in south Kerala. In the coastal and backwater

[1] Contribution No. 558 of CPCRI, Regional Station, Kayangulam.

tracts of Quilon and Alleppey districts of Kerala State, the aggregate natural parasitism by *T. pupivora* was 1.27% (11/864 pupae) in 1979 and 2.0% (15/754 pupae) in 1980. The mean aggregate parasitism thus worked out to 1.6% only (26/1618 pupae) during the two years.

The inefficiency of *T. pupivora* stems from its (a) low searching and dispersal ability; (b) low tolerance for non-optimal temperatures; (c) poor sense of discrimination between parasitised and unparasitised host which leads to superparasitism, and (d) inability to compete with other pupal parasites such as *Brachymeria* spp. As the host pupae remained within cocoons in silken galleries the parasite requires more time to locate the host and oviposit in it. It has to penetrate through into the silken galleries and then bite and make a small hole in the cocoon before getting access to the host pupa for oviposition. Its ovipositor is very short and, as such, it cannot oviposit standing on the cocoon. The parasite takes six to several hours to reach the host pupa. In the field it cannot oviposit more than one or two host pupae. The parasite lays majority of its eggs in the first host itself it encounters, irrespective of the size of the host pupa.

The chalcidids *Brachymeria nosatoi* Habu and *B. nephantidis* Gahan suppressed the population of *T. pupivora* to a considerable extent. This is an important factor responsible for limiting the efficiency of *T. pupivora* in the southern region of Kerala, where natural parasitism by *Brachymeria* spp. is quite high. JOY & JOSEPH (1977) also observed that parasitism by *T. pupivora* in southern Kerala was low, i.e. 0—11%, with a mean of less than 1%, while in north Kerala it was higher (14%). They observed that parasitism by *B. nosatoi* was only 2.2% in north Kerala, while it was 19.3% in the southern region. As competition from *Brachymeria* spp. was less intense in north Kerala in view of their low population levels the intensity of parasitism by *T. pupivora* can naturally be more in those areas.

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BRIEF COMMUNICATION

RELATIVE TOXICITY OF SOME INSECTICIDES TO ADULTS OF *CHELONUS BLACKBURNI* CAM.

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Phosalone is found to be least toxic to the adult *Chelonus blackburni* followed by carbaryl, endosulfan, monocrotophos and fenvalerate.

(Key words: relative toxicity, parasitoid, *Chelonus*)

The possibility of using *Chelonus blackburni* CAM. (Hym:Brac) as egg/larval parasitoid for control of the cotton bollworms *Earias vitella* F., *Heliothis armigera* HBN. and *Pectinophora gossypiella* (SAUNDERS) has been indicated (SWAMIAPPAN & BALASUBRAMANIAN, 1980). In order to find out the relative toxicity of different pesticides used for cotton pest control to adults of *C. blackburni* dosage mortality relation between the toxicants and the parasitoid and LC_{50} of the insecticides were worked out, the adults of *C. blackburni* were anaesthetised by ether. Ten such anaesthetised adults were sprayed with one ml of the graded concentration of the insecticides (see Table 1) through the 'Potters Tower' at 1.25 kg/cm^2 pressure. Water was used as control. There were three replications. The treated adults were

TABLE 1. Relative Toxicity of insecticides to adults of *C. blackburni*.

Insecticides	LC 50	Fiducial limits	Regression equation	Chi square value	Relative toxicity
Phosalone (Zolone 35% EC)	0.1134	0.11328 0.11352	Y = 6.46 X−12.33	−10.2945	1.47
Carbaryl (Sevin 50% WP)	0.07702	0.07686 0.07718	Y = 2.231 X+ 0.488	0.8811	1.00
Endosulfan (Thiodan 35% EC)	0.04802	0.04788 0.04816	Y = 3.239 X− 3.535	1.336	0.62
Monocrotophos (Nuvacron 40% EC)	0.04062	0.04049 0.04075	Y = 3.4368 X− 3.852	−3.262	0.53
Fenvalerate (Sumicidin 20% EC)	0.03166	0.03133 0.03219	Y = 7.0435 X−16.47	0.3008	0.41
Dimethoate (Rogor 30% EC)	0.01062	0.01042 0.01082	Y = 3.1215 X− 2.910	0.8104	0.14

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kept in small (lid perforated) containers (7 × 7.5 cm at 27°C ± 3°C) and 50—60 per cent relative humidity. Mortality counts were taken 12 hours after spraying. The data were analysed by probit method (FINNEY, 1952).

Phosalone was found to be the least toxic to adult *C. blackburni* followed by carbaryl, endosulfan, monocrotophos, fenvalerate and dimethoate in that order of ascending toxicity (Table 1). Phosalone was reported earlier to have low toxicity to *Trichogramma* (NAVARAJAN PAUL *et al.*, 1979). Endosulfan was reported to be safe to beneficial insects (RECHAV, 1974; NAVARAJAN PAUL *et al.*, 1979). Fenvalerate, which was highly toxic to the parasite in the present studies, was reported to be safe by others (WADDILL, 1978). Dimethoate, though primarily a systemic poison showed high contact toxicity to the parasitoids.

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BRIEF COMMUNICATION

EFFECT OF EGG PLANT SEEDLING ROOT DIP FOR THE CONTROL OF SUCKING PESTS

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Five insecticides, acephate, methamidophos (Tamaron), FMC 35001, monocrotophos and chlorpyriphos alone and in combination with urea 1% and wet cow dung 2% (w/w) were evaluated for efficacy as seedling root dips against aphids *(Aphis gossypii)*, mealy bugs *(Centrococcus insolitus)* and mites (*Tetranychus cinnabarinus*) of egg plant. Monocrotophos 0.05 per cent appeared to be the most potential insecticide which could be utilized in prophylactic seedling root dip for affording protection to the crop for a period of 21 days after transplanting, during which period cultivators in the conventional way would have resorted to one foliar spray.

(Key words: egg plant, seedling root dip, insecticides, control of sucking pests)

Egg plant *(Solanum melongena* L.) is valued for its immature fruits. It is nutritious, and much preferred because it is cheap and can be grown throughout the year in India. In its early stage the crop is affected by a few important sucking pests like cotton aphid, *Aphis gossypii* G., mealy bug, *Centrococcus insolitus* (Gr.) and mite, *Tetranychus cinnabarinus* B. causing crinkling and mottling of leaves and stunting of plants resulting in loss of plant vigour. In order to minimise the use of insecticides the seedling root dip treatment was tried in this experiment.

Two experiments were conducted to evaluate five different insecticides, either alone or in combination with urea 1% or wet cowdung 2% (w/w) for their efficacy as seedling dips (Table 1). Root portions of 30 day old brinjal seedlings (cultivar *MDU 1*) were kept immersed for 3 hours in these solutions. Dipping roots for similar period in water formed the check. The seedlings were transplanted in pots and kept in a wire mesh cage to prevent access to other pests, parasitoids and predators. Onto each plant, upon establishment (3 days after transplanting), ten adults of *Aphis gossypii*, *Centrococcus insolitus* or *Tetranychus cinnabarinus* were introduced with a camel hair brush. Observations on the mortality of pest were recorded at 24 hr, 72 hr, 1 week, 2 weeks and 3 weeks after introduction. After each observation, the number of insects were maintained constant by fresh introduction in case of mortality and by brushing out the excess in case of multiplication of the pest. The experiment was conducted in randomized block design with two replications. Because there was no mortality in some observations, the values of percentage mortality were added with 0.1 and transformed into corresponding angles (arc sine $\sqrt{\text{percentage}}$) for statistical interpretation.

Data are presented in Table 1. In both the experiments root dip in monocrotophos 0.05 per cent afforded good protection against all the three pests *viz.*,

TABLE 1. Mortality (%) of sucking pests in brinjal seedlings dipped in some insecticides alone and with urea (Mean of 10 observations).

Root dip in:	*Tetranychus cinnabarinus*		*Centrococcus insolitus*		*Aphis gossypii*	
	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
1. Acephate 0.15%	20.00 (21.42)	19.00 (21.17)	43.00 (37.51)	44.00 (39.54)	63.00 (54.96)	84.00 (73.21)
2. Methamidophos 0.06% (Tamaron)	64.00 (56.50)	56.00 (48.94)	51.00 (44.43)	59.00 (51.84)	89.00 (77.68)	90.00 (78.29)
3. FMC 35001, 0.048%	33.00 (33.16)	48.00 (44.07)	42.00 (37.59)	59.00 (50.48)	92.00 (81.02)	92.00 (80.79)
4. Monocrotophos 0.05%	89.00 (75.28)	94.00 (80.02)	70.00 (58.81)	86.00 (73.14)	92.00 (79.55)	95.00 (82.87)
5. Chloropyriphos 0.04%	19.00 (19.52)	34.00 (34.23)	35.00 (30.78)	23.00 (23.33)	28.00 (26.41)	40.00 (37.60)
6. Acephate 0.15% + urea 1%	13.00 (14.82)	13.00 (14.82)	33.00 (30.95)	52.00 (43.70)	36.00 (34.08)	41.00 (38.90)
7. Methamidophos 0.06% + urea 1%	60.00 (52.78)	80.00 (68.37)	42.00 (40.77)	67.00 (59.79)	89.00 (77.71)	89.00 (77.62)
8. FMC 35001, 0.048% +Urea 1%	23.00 (24.89)	17.00 (22.04)	41.00 (38.21)	65.00 (54.77)	93.00 (82.86)	96.00 (84.70)
9. Monocrotophos 0.05% + Urea 1%	78.00 (63.95)	85.00 (72.56)	76.00 (64.02)	85.00 (75.26)	96.00 (84.85)	96.00 (84.70)
10. Urea 1%	0.00 (1.81)	0.00 (1.81)	2.00 (4.29)	0.00 (1.81)	0.00 (1.81)	0.00 (1.81)
11. Chlorpyriphos 0.04% + Urea 1%	9.00 (10.87)	11.00 (12.37)	17.00 (19.46)	20.00 (21.51)	13.00 (12.22)	25.00 (22.05)
12. Acephate 0.15% + Cowdung 2%	12.00 (15.26)	19.00 (17.35)	16.00 (17.47)	16.00 (19.15)	54.00 (47.93)	81.00 (68.19)
13. Methamidophos 0.06% + Cowdung 2%	41.00 (38.60)	75.00 (62.31)	41.00 (38.60)	66.00 (57.89)	63.00 (53.50)	85.00 (74.99)
14. FMC 35001, 0.048% + Cowdung 2%	19.00 (18.51)	14.00 (17.89)	19.00 (18.51)	63.00 (55.69)	89.00 (78.94)	90.00 (79.54)
15. Monocrotophos 0.05% + Cowdung 2%	68.00 (60.31)	70.00 (61.78)	68.00 (60.31)	76.00 (64.37)	89.00 (77.47)	96.00 (84.85)
16. Chlorpyriphos 0.06% + Cowdung 2%	3.00 (6.83)	3.00 (6.83)	3.00 (6.83)	19.00 (19.75)	19.00 (19.61)	29.00 (30.80)
17. Cow dung 2%	0.00 (1.81)	0.00 (1.81)	0.00 (1.81)	1.00 (3.48)	13.00 (13.63)	2.00 (4.29)
18. Control	0.00 (1.81)	0.00 (1.81)	0.00 (1.81)	0.00 (1.81)	0.00 (1.81)	0.00 (1.81)
C D = (P = 0.05)						
Between treatments	13.73	4.67	11.46	6.80	9.40	8.88

Figures in parantheses are angular transformed values.

T. cinnabarinus, *C. insolitus* and *A. gossypii*. Next to monocrotophos, methamidophos + urea 1% treatment gave good protection in both the experiments. The effectiveness of methamidophos + urea as seedling dip against sucking pests is in corroboration with the result obtained by DHAKSHINAMOORTHY (1980). Acephate 0.15% and FMC 35001, 0.048% per cent alone and in combination with urea and cowdung was found intermediate in effectiveness. Chlorpyriphos 0.04 per cent either alone or in combination with urea or cowdung was found ineffective in both of the experiments in controlling any of these pests. Effectiveness of chlorpyriphos as seedling dip in rice (AZAM & SHAMIM BANO, 1977; SHARNNAGAT *et al.*, 1980) and its ineffectiveness in egg plant may be due to the kind and physiology of the crop involved.

In general, reduction in mortality was noticed when insecticides were combined with urea and cowdung, but the differences were not significant. This may perhaps be attributed to formation of ammoniacal compounds, when the pesticides are combined with urea, resulting in reduced activity of the toxic ingredient (RAMAN & KRISHNAMOORTHY 1975), After 21 days of treatment, the mortality of the pest was not high even in the effective treatments, but the pest population was not allowed to increase.

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BRIEF COMMUNICATION

PERSISTENCE OF TOXICITY OF CONTACT INSECTICIDE RESIDUES ON CEMENT SURFACE TO STORAGE PESTS

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Of the ten insecticides tested, the residues of isofenphos, fenitrothion, fenthion and phoxim had the maximum toxicity and persistence when sprayed as emulsions on cement surface, to adults of *Rhizopertha dominica* Fab. and *Tribolium castaneum* Hbst.

(Key words: insecticide residues, cement surface, toxicity, *Rhizopertha dominica*, *Tribolium castaneum*)

Different species of insect pests infest stored commodities. One of the basic recommendations for the storage of materials free from such infestations is keeping the godown free from the insects by use of insecticides. But no information is available on the relative efficacy of different insecticides for this purpose. The present studies were hence undertaken in which persistent toxicity of residues of ten insecticides on cement surface to the two major storage pests—the red flower beetle, *Tribolium castaneum* HBST. and the lesser grain borer, *Rhizopertha dominica* FAB. was ascertained.

Adults of *T. castaneum* and *R. dominica* were reared on wheat flour and wheat grains respectively. Cement tiles of 20 cm^2 were used to simulate godown walls and floors. The different insecticides (as detailed in Table 1) were sprayed @ 5 ml/

TABLE 1. Persistent toxicity of insecticides sprayed on cement surface to storage pests.

Insecticides and concentration	*R. dominica*			*T. castaneum*		
	Persistence (days)	Average mortality (%)	PT index	Persistence (days)	Average mortality (%)	PT index
BHC 0.2%	30	58.33	1749.99	30	28.00	840.00
Carbaryl 0.2% (Sevin)	90	69.22	6229.80	105	33.64	3532.20
Fenitrothion 0.1% (Folithion)	90	63.92	5702.80	155	78.88	12226.40
Fenthion 0.1% (Lebaycid)	90	46.5	4185.00	90	79.82	7184.00
Malathion 0.5%	60	62.23	3734.00	30	28.87	866.10
Phosalone 0.2% (Zolone)	60	49.18	2950.80	60	12.78	766.80
Quinalphos 0.1% (Ekalux)	30	72.63	2178.99	90	64.53	5807.77
Phoxim 0.1% (Baythion)	60	69.4	4164.00	90	58.9	5301.00
Isofenphos 0.2% (Oftanol)	90	70.83	6375.00	155	72.66	12262.30
Fenvalerate 0.05% (Sumicidin)	30	28.0	840.00	1	36.7	36.70

tile and dried in shade. Toxicity of the insecticide residues on the tiles was assessed by confining the test insects on the sprayed tiles under inverted petridishes fixed on the tile surface with mud plaster at intervals of 15 days and mortality counts taken 24 hours after exposure to the treated surface. The tiles were kept under godown conditions. Persistence of the toxicity of the insecticides was determined in terms of PT indices, calculated following the method of PRADHAN (1967) where 'P' is the period up to which toxicity persisted and 'T' is the average per cent mortality.

Results presented in Table 1 show that with reference toxicity and persistence of the insecticide residues deposited on cement surface to both the test insects, the insecticides which ranked high were isofenphos, fenitrothion, fenthion and phoxim. These insecticides can hence be recommended for spraying the walls and floors as a measure against insect infestation in storages. The sprayings may be repeated at 3 months intervals to ensure a continued prevention of the infestation. Phoxim residues had been reported to be effective against storage pests by GIRISH *et al.* (1973) and PANDEY *et al.* (1979) when applied on cement concrete slabs.

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BRIEF COMMUNICATION

SUSCEPTIBILITY OF DIFFERENT PESTS AND PLANTS TO INFECTION BY *FUSARIUM MONILIFORMAE* VAR. *SUBGLUTINANS*

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Studies conducted on the susceptibility of some of the common vegetable pests of *Fusarium moniliformae* var. *subglutinans* showed that *Mylabris pustulata* and *Aulocophora* sp. were highly susceptible to infection by the fungus under laboratory conditions. The pathogen did not harm cotton, tomato, bitter gourd, brinjal and snake gourd.

(*Key words: Fusarium moniliformae* var. *subglutinans*, susceptibility of pests and plants)

JACOB *et al.* (1978) reported *Fusarium moniliformae* var. *subglutinans* as a pathogen of the epilachna beetle *Henosepilachna vigintioctopunctata* (F.). Studies were undertaken to assess the pathogenicity of this fungus to other common vegetable pests and some plants.

Fungal culture obtained from diseased grubs of the beetle was maintained in Richards medium. The beetles under test were released on the respective host plant shoots caged in chimneys with moist cotton wool to ensure high humidity and sprayed with a concentrated suspension of the spores collected from 9-day-old cultures. The caterpillars tested were inoculated by allowing them to crawl for one hour over heavily sporulated 9-day-old cultures and reared on respective host plant shoots enclosed in hurricane chimneys. Mortality

TABLE 1. Infectivity of *F. moniliformae* var. *subglutinans* to different insects.

Test insect	Stage of insects treated	Per cent mortality of treated insects	Average time taken for death in days	Infection
Aulacophora sp.	Adults	100	4	+
Mylabris pustulata	,,	100	5	+
Leucinodes orbonalis	Larvae	40	6	+
Anadevidia peponis	,,	35	6	+
Sylepta derogata	,,	45	5	+
Antoba olivacea	,,	0	–	–
Hymenia recurvalis	,,	20	5	+
Spodoptera litura	,,	0	–	–
Earias vitella	,,	40	6	+

Each experiment was replicated three times using 10 insects per replication. No mortality in control.

of the insects was observed daily till all were dead or pupated as the case may be. Pathogenicity was confirmed by re-isolating the fungus from the dead specimens.

Results presented in Table 1 show that *F. moniliformae* var. *subglutinans* was highly infective to *Aulacophora* sp. and *Mylabris pustulata*. Larvae of *Leucinodes orbonalis*, *Anadevidia peponis*, *Sylepta derogata* and *Earias vitella* were moderately susceptible and that of *Hymenia recurvalis* showed low susceptibility. The fungus was not infective to larvae of *Antoba olivaceae* and *Spodoptera litura*.

Pathogenicity of the fungus to some of the crop plants was assessed by: (1) soil treatment in which surface soil in the pots was mixed with 25 ml of the spore suspension and seedlings planted after two hours of application. Soil mixed with 25 ml sterile distilled water served as control. (2) Seed treatment in which 25 g of the seeds were soaked in spore suspension for 24 hours and sown in flower pots at a rate of two seeds per pot. (3) Root treatment in which root tips of fifteen day old seedlings were cut and kept immersed in the spore suspension for 2 hours and planted in pots at a rate of one seedling per pot. (4) Leaf treatment in which injury was made on leaves by pin pricks and culture bits placed on the injured spots and covered with moist cotton wool. All the above experiments were replicated 4 times for each crop. Observations were made daily on the condition of the plants till harvest.

None of the plants inoculated with the fungus took infection and these included cotton, tomato, bitter gourd, brinjal and snake gourd plants.

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BRIEF COMMUNICATION

STUDIES ON RELATIVE SUSCEPTIBILITY OF STAGES OF *HENOSEPILACHNA VIGINTIOCTOPUNCTATA* (F) TO INFECTION BY *FUSARIUM MONILIFORMAE* VAR. *SUBGLUTINANS* AND ITS USE IN THE CONTROL OF THE PEST

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Studies conducted to assess the relative susceptibility of different stages of *H. vigintioctopunctata* to infection by *F. moniliformae* var. *subglutinans* showed that third and fourth instar grubs were more susceptible than first and second instar grubs. Pupae were less susceptible than larvae and adults the least susceptible of all the stages. The pathogen was effective at high concentrations in controlling the pest on bitter gourd.

(Key words: Henosepilachna vigintioctopunctata, control by *Fusarium moniliformae* var. *subglutinans*)

Fusarium moniliformae var. *subglutinans* was observed infecting grubs and adults of epilachna beetle, *Henosepilachna vigintioctopunctata* (Coleoptera : Coccinellidae) by JACOB *et al.* (1978). Results of studies conducted to assess the relative susceptibility of different stages of the beetle to infection by the pathogen and those of a cage test undertaken to explore the suitability of the pathogen as a controlling agent under field conditions are reported in this paper.

For studies on susceptibility of different stages, spore suspensions prepared from a 9 day old culture of *F. moniliformae* var. *subglutinans* at a concentration of 4.5×10^5 conidia/ml were used for inoculation. Terminals of bitter gourd vines were kept turgid by keeping the lower

TABLE 1. Mortality of stages of *H. vigintioctapunctata* inoculated with *F. moniliformae* var. *subglutinans*.

Instars	Cumulative per cent mortality at intervals* (days)				
	1	2	3	4	5
First larval	36.67	76.67	86.67	96.67	96.67
Second ,,	26.67	60.00	76.67	90.67	90.67
Third ,,	44.33	76.67	96.67	100.00	100.00
Fourth ,,	50.00	76.67	93.33	100.00	100.00
Pupal	30.00	56.67	66.67	80.00	80.00
Adult	23.33	43.33	63.33	70.00	76.67

* Mean of 3 replications of 15 insects each.

TABLE 2. Mortality of *H. vigintioctopunctata* on bitter gourd sprayed with spores of *F. moniliformae* var. *subglutinans*.

Concentration of spores (conidia/ml)	Cumulative per cent mortality in days*					
	2	3	4	5	6	7
3.0×10^5	4.44	10.00	21.11	32.22	42.22	50.00
4.5×10^5	11.67	16.67	33.33	41.11	53.33	67.78
6.0×10^5	20.00	34.45	46.67	63.33	74.45	83.33
7.5×10^5	25.50	44.44	64 33	80.00	88.89	96.67

* Mean of 3 replications each.

end dipped in water in specimen tubes closed with cotton wool. The specimen tubes with twigs were enclosed in glass chimneys. Moist cotton wool was placed at the bottom of chimneys to ensure high humidity. Grubs, pupae or adults as the case may be were exposed on the twigs and sprayed with spore suspension and mortalities recorded daily.

The efficacy of pathogen under field conditions was tested with four concentrations of spore suspensions *viz.* 3×10^5, 4.5×10^5, 6×10^5 and 7.5×10^5 conidia/ml. Grubs, pupae and adults of the beetle were exposed on 30 to 40 days old bitter gourd plants grown in pots (30 cm diameter) and enclosed in polythene cages. Spore suspensions were applied 2 days after release of the insect with 50 ml of the spore suspension per cage using a glass atomizer. Insects sprayed with 50 ml distilled water served as control. The caged pots were kept under field conditions. All the stages were susceptible to infection by the pathogen eventhough they varied in their susceptibility (Table 1).

The third and fourth larval instars showed the maximum susceptibility to the infection giving cent per cent mortality in 4 days of the infection. First and second instar larvae were less susceptible than the third and fourth while the pupa ranked below all the larval instars in the decreasing order of susceptibility; the adults were the least susceptible. KRISHNAMOORTHY & NAIDU (1971) had reported that all the stages of epilachna beetle were susceptible to infection by *Aspergillus flavus*. In the field cage test the fungus at the cencentration of 7.5×10^5 conidia/ml caused 96.67 per cent mortality of the insect within 7 days while the three lower doses gave less mortalities of 50.0 to 83.33 per cent (Table 2).

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PATHOGENICITY OF CERTAIN SEROTYPES OF *BACILLUS THURINGIENSIS* BERLINER AGAINST *ACHAEA JANATA* L.

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Attempts were made to study the comparative pathogenicity of six varieties of *Bacillus thuringiensis* against *Achaea janata*, the castor semilooper. Production studies on nutrient broth revealed that the variety, *kurstaki* produced more spores per litre of the medium followed by varieties *berliner*, *galleriae*, *entomocidus* and *aizawai*. Variety *sotto* produced the least number of spores. Endotoxin production was more in varieties, *galleriae* and *sotto*, whereas it was less in varieties *aizawai* and *entomocidus*. Varieties *kurstaki* and *berliner* produced moderate level of endotoxin. Comparative bioassay of spore + endotoxin and predissolved endotoxin indicated that variety *kurataki* was more pathogenic followed by varieties *galleriae* and *berliner*. Varieties *sotto*, *entomocidus* and *aizawai* or their endotoxin alone did not show pathogenicity to 5th instar larvae of *A. janata*.

(Key words: Bacillus thuringiensis, kurstaki, sotto, galleriae, entomocidus, aizawai, Achaea janata, endotoxin).

INTRODUCTION

Microbial insecticides, because of their specificity and non-toxicity or pathogenicity to non-target animals, are ideal biological control agents. In addition they are easier to use than the classical biological control agents i.e., parasites and predators, because the grower can use them, as he would, chemical insecticides without any major change in agricultural equipment and procedure (IGNOFFO *et al.*, 1974). A broad spectrum but selective pathogen like *Bacillus thuringiensis* BERLINER would be an ideal agent for use in the pest management programmes.

JAQUES & FOX (1960) first studied the differential pathogenicity of varieties of *B. thuringiensis* against *Pieris rapae*. Further studies on this aspect were conducted by HEIMPEL (1961) against saw flies, SMIROOF (1965) against *Choristoneura fumiferana*, BROERSMA & BUXTON (1967) against *Trichoplusia ni*, BURGES (1967) and ROGOFF *et al.* (1969) against *Galleria mellonella*, ANGUS & NORRIS (1968) against *Bombyx mori*, YAMVRIAS & ANGUS (1969, 1970) against *Tineola bisettiella*, ROSSMOORE *et al.* (1970) against *Hemerocampa leucostigma*, LARSON & IGNOFFO (1971) against *Alsophila pometaria*, RIDET (1973) against *Lymantria dispar* and ROIG *et al.* (1975) against *Prays oleae*.

Similarly differential toxicity of d-endotoxin from various varieties of *Bacillus thuringiensis* was studied by ANGUS (1967) against *Bombyx mori*, PENDLETON (1969) against *Philosamia cynthia*, YAMVRIAS & ANGUS (1969) against *T. bisettiella* and GALOWALIA *et al.* (1973) against *P. brassicae* and *B. mori*.

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It was well demonstrated that different varieties of *B. thuringiensis* exhibited different levels of pathogenicity in various insect species and also that in the same host species different varieties of *B. thuringiensis* exhibited different degrees of pathogenicity. However, very little has been done either on the differential pathogenicity of varieties of *B. thuringiensis* or their toxins against the various crop pests in India. Such studies will be of importance in selecting a most virulent variety against a particular pest. In the present studies the differential pathogenicity and toxicity of *B. thuringiensis* varieties and their d-endotoxin were investigated with castor semilooper, *Achaea janata* as a test insect.

MATERIALS AND METHODS

A healthy laboratory culture of *A. janata* was maintained on castor leaves at 28°C. The bacterium was grown on nutrient broth medium according to YAMVIRIAS & ANGUS (1970). The biomass containing vegetative cells and spores was separated from the medium by centrifuging it at 12,000 g (FAUST *et al.*, 1974). The pelleted material was dried in watch glasses kept in a desiccator containing fused anhydrous calcium chloride. Spore counts of each variety were made with the use of haemocytometer after serial dilution, under phase contrast microscope. This dried powder was either used for experimentation or for the extraction of soluble endotoxin. Partially purified endotoxin was obtained as per the method of FAUST (1974). The endotoxin was quantified on the basis of protein content according to LOWRY *et al.* (1951).

Comparative bioassay of six varieties of *B. thuringiensis* with spores + endotoxin and predissolved endotoxin was carried out against 5th instar larvae of *A. janata*. In all the bioassay studies, serial dilutions of the preparation were made in 0.1% Triton-X distilled water. On 2.5 cm diameter discs of castor leaves 40 to 80 µl of the dilution was applied and dried under fan. These treated leaf discs were placed in specimen tubes (one disc/tube) and 3hr prestarved 5th instar larvae were released (one larva/tube). These specimen tubes were plugged with cotton. The larva which consumed at least a part of the disc was kept for further observations. Fresh food was offered at 24 hr interval. A total of 20 to 30 larvae were fed on discs of each concentration. Adequate number of larvae was kept as control. Mortality data were recorded upto 96 hr. The LC_{50} and LT_{50} values were calculated by probit analysis (FINNEY, 1977).

RESULTS AND DISCUSSION

The data on the biomass, the spore count and the endotoxin content of the different varieties of *B. thuringiensis* are presented in Table 1.

It can be inferred from the data in Table 1 that all the varieties of *B. thuringiensis* were able to grow in the nutrient broth. Varieties *berliner*, *kurstaki*, *sotto* and *aizawai* yielded double the quantity of biomass as compared to *galleriae* and *entomocidus*. Similarly PANA-BERATLEIF (1968) obtained more biomass from *B. thuringiensis* variety *berliner* as compared to *galleriae*.

Variety *kurstaki* yielded more spores as compared to others. DULMAGE (1970) also reported that the variety *kurstaki* was a high spore yielder. Except the variety *sotto* which was a poor yielder of spore, other varieties produced more or less equal number of spores per litre of the medium. ROGOFF *et al.* (1969) and DULMAGE (1970) also reported poor yield of variety, *sotto* in comparison to other varieties when these were grown on endotoxin (fish meal + starch) and exotoxin (corn steep + cotton seed oil meal + cane beet molasses) promoting and nutrients plus tryptone or proflo based media. In their experiments *aizawai* was the highest yelder. In the present studies the medium on which varieties of *B. thuringiensis* was grown was the nutrient broth. The poor yield of *aizawai* might be due to deficiency of the medium.

TABLE 1. Yield of different varieties of B. *thuringiensis.*

Sl. no.	Serotype	Name of the variety of *B. thuringiensis*	Biomass obtained (gm/lit)	Spores litre of the medium (1×10^{10})	Proteinaceous endotoxin content (μg/100 μl)
1	1	*berliner*	0.37	3.96	490.0±14.1 a, d
2	3	*kurstaki*	0.40	5.03	470.0±14.1 a, b
3	4	*sotto*	0.38	2.43	555.0±28.2 e, d
4	5	*galleriae*	0.16	3.85	562.5±67.0 d
5	6	*entomocidus*	0.17	3.63	235.5±31.8 e
6	7	*aizawai*	0.37	3.05	327.5±24.7 f
		C D	0.08	1.07	± Means followed by the same letters are not significant 't'—test

Remaining varieties i.e., *berliner*, *galleriae* and *entomocidus* were more or less equal spore yielders.

The endotoxin could be estimated by three methods *viz.*, by counting the number of crystals, estimation of predissolved protein or by its toxicity to test insects. In the present investigation the quantity of predissolved endotoxin was determined by Lowry's method and the toxin was assayed against *A. janata.*

Variety, *galleriae* produced the highest quantity of predissolved endotoxin which may be due to the fact that this variety had higher spore count per gram of biomass. DULMAGE & BARJAE (1973) also reported higher endotoxin content of variety, *galleriae.*

In varieties, *berliner* and *kurstaki* the spore per gram and the quantity of endotoxin were almost equal. YAMVRIAS & ANGUS (1970) also observed equal spore to crystal ratio.

Normally in the varieties of *B. thuringiensis* the ratio of spore to crystal is 1:1. But in the present studies, the lower spore count and higher protein content of varieties, *sotto* and high spore count and low protein content of *entomocidus* suggest that the spore to crystal ratio of 1:1 was not applicable. The discrepency in the amount of pre-dissolved endotoxin might be due to size variation in crystals because of varietal differences as has been pointed out by PENDLETON (1969). DULMAGE (1970, 1971) rightly pointed out that the quantity of endotoxin produced in the fermentations could not be predicted from the variant selected, the growth and sporulation observed or the fermentation medium used.

In case of variety, *aizawai* the spore count as well as the endotoxin was found to be low compared to other varieties.

The results obtained in the bioassay studies of spore + endotoxin of six varieties of *B. thuringiensis* are presented in Table 2.

On the basis of LC_{50} values it can be concluded that variety, *kurstaki* was superior and was 1.5 and 3.8 times more toxic than the varieties, *galleriae* and *berliner* respeetively. The varietics, *sotto*, *aizawai* and *entomocidus* did not show any

TABLE 2. Bioassay of spore + endotoxin of *B. thuringiensis* against the 5th instar larvae of *A. janata*.

S. no.	Serotype	Variety of *B. thuringiensis*	LC 50 (spores/ml)	Fiducial limit (spores/ml)		b value (slope)	LT 50 (hr)
				upper	lower		
1.	1	*berliner*	103.7×10^7	233.4×10^7	46.1×10^7	1.24	75.6
2.	3	*kurstaki*	27.4×10^7	51.5×10^7	14.5×10^7	1.52	41.9
3.	5	*gallerai*	41.9×10^7	107.7×10^7	16.4×10^7	1.27	36.3
4.	4	*sotto**	18.6×10^7	43.3×10^7	$8\ 10^7$	1.672	—

* against fourth instar.

pathogenicity against 5th instar larvae of *A janata*.

Present results are quite comparable with the results obtained by ROGOFF *et al.* (1969). During the examination of pathogenicity of 31 strains, grown on two synthetic media, they observed that variety *aleati* (from which variety *kurstaki* was isolated) ranked first followed by varieties *galleriae* and *thuringiensis* against *G. melonella*, *T. ni.*, *Heliothis zea* and *Musca domestica*. In their experiments almost all isolates of varieties *sotto*, *aizawai* and *entomocidus*, grown on media promoting endotoxin and exotoxin did not exhibit any pathogenicity against all the 5 insects tested.

More or less similar pattern of results were also noticed by YAMURIAS & ANGUS (1970). They found that the varieties in order of toxicities were *alesti*, *thuringiensis*, *entomocidus*, *sotto* and *galleriae* against *C. fumiferana*. ROSSMOORE *et al.* (1970) also reported the pathogenicity of these varieties with a change in the order of *thuringiensis*, *sotto*, *entomocidus*, *alesti* and *aizawai* against *H. leucostigma*. However, varieties *entomocidus* and *sotto* which were less pathogenic to some insects were found to be highly pathogenic to *B. mori* (ANGUS & NORRIS, 1968).

The slope value of dosage mortality curve inhibited the degree of variability in the insect population tested, lower the slope value greater the variability. Normally, the slope values of the bioassay studies with the microorganisms are very low (BURGES & THOMSON, 1971). In the present investigations for the three varieties of *B. thuringiensis* the slope values were : *berliner* 1.235, *kurstaki* 1.522 and *galleriae* 1.273 indicating a uniform susceptibility of the insect population to the bacteria.

When low pathogenic varieties were tested against earlier instar larvae of the pest, it was observed that only *sotto* was pathogenic to the 4th instar, the LC_{50} value being 18.62×10^7 spores/ml. This differential pathogenicity to different instars may be due to the maturity of the larvae which increased the tolerance. MORRISON & PERRON (1963) found that susceptibility of *G. melonella* to *B. thuringiensis thuringiensis* decreased from first to fifth instar. However, ROOSMOORE *et al.* (1970) found that there was no difference in susceptibility to *B. thuringiensis* among first through fifth instar larvae of *H. leucostigma*.

The LT_{50} values for the three pathogenic varieties were: *berliner* 75.6 hr,

TABLE 3. Bioassay of predissolved endotoxin of *B. thuringinesis* aginst 5th instar larvae of *A. janata*.

S. no.	Serotype	Name of the variety of *B. thuringiensis*	LC 50 µg/ml	Fiducial limit µg/ml		b value (slope)
				upper	lower	
1.	1	*berliner*	5288.0	9097.0	3074.0	2.00
2.	3	*kurstaki*	634.0	1290.0	312.0	1.74
3.	5	*galleriae*	1704.0	4414.0	658.0	1.03

kurstaki 41.9 hr and *galleriae* 36.3 hr. The difference in LT_{50} values of *galleriae* and *kurstaki* is not significant but variety *berliner* recorded significantly higher value.

The lower LT_{50} value in variety *galleriae* compared to *kurstaki* could be due to the fact that the spore concentration per unit weight was more in the case of *galleriae*. Further, BROERSMA & BUXTON (1967) while studying the histopathology of various varieties in *T. ni* observed that the progression of pathology of the larvae which fed on *B. thuringiensis-galleriae* was more rapid than in the larvae feeding on any of the other bacteria tested. The quicker action of varieties *galleriae* and *kurstaki* over *berliner* is probably due to the primary or secondary action of proteases as pointed out by LECADET & MARTOURET (1962) in their studies with *berliner* and *anduze* strains.

The results of bioassay of endotoxin with different varities of *B. thuringiensis* against 5th instar larvae of *A. janata* are presented in Table 3.

In the comparative bioassay with predissolved endotoxin only three varieties against *A. janata*, the other three varieties ie., *sotto*, *entomocidus* and *aizawai* did not exhibit any toxicity. The LC_{50} values for the three toxic varieties were: *berliner* 5288, *kurstaki* 634 and *galleriae* 1704 µg/ml.

GALOWALIA *et al.* (1973) in their studies with *P. brassicae* found that the endotoxin of varieties, *thuringiensis*, *galleriae* and *entomocidus* were equally toxic and the variety *sotto* too weakly toxic to record its potency. In studies with pure endotoxin PENDLETON (1969) and YAMVRIAS & ANGUS (1969) also reported the superiority of variety *thuringiensis* over varieties *entomocidus* and *sotto* while working on *P. cynthia* and *T. bissettiella* respectively.

ANGUS (1967) and GALOWALIA *et al.* (1973) observed that the endotoxin of varieties, *entomocidus*, *sotto* and *aizawai* was more toxic and the endotoxin of *thuringiensis* was least toxic amongst the several varieties tested against *B. mori*.

In the present investigation the order of toxicity for both spore + crystal and predissolved endotoxin was: *kurstaki* followed by *galleriae*, followed by *berliner* followed by *sotto* (4th instar). Other two varieties i.e., *aizawai* and *entomocidus* were not pathogenic to *A. janata*.

Besides the inherent varietal differences the other point to consider is the nature and property of the endotoxin. GRIGAROVA *et al.* (1967) reported that the bipyramidal and biprismatic shapes of the crystals also contributed to differential toxicity. It is also reported that the endotoxin predissolved either by gut juices

or by NaOH revealed different antigenic properties (PENDLETON, 1968). This brings out the fact that the crystal toxin of different varieties had different toxic moieties and the toxicity was predominantly a host response phenomenon.

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STUDIES ON THE DAMAGE AND CHEMICAL CONTROL OF CHAFER BEETLES ON GRAFTED BER (*ZIZYPHUS MAURITIANA* LAMK.) CULTIVARS

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Fifteen *ber* cultivars were evaluated for the loss caused by the chafer beetles *Holotrichia consanguinea* Bl., *Anomala bengalensis* Bl. and *Adoretus* sp. Cultivar *Aliganj* suffered least damage in the field. Maximum leaf area was consumed of *Gola* cultivar and the highest per cent leaf area loss was observed in *Jogia* cultivar. No significant difference was observed in the consumption of leaves by the three beetle species studied. Methyl parathion, fenitrothion and quinalphos were found to be very effective against *H. consanguinea* Bl.

(*Key words:* chafer beetles, *Zizyphus*, cultivar evaluation, chemical control)

Ber, *Zizyphus mauritiana* LAMK. is one of the preferred host plants of the chafer beetles viz., *Holotrichia consanguinea* BL., *H. insularis* BL., *Anomala bengalensis* BL., *Adoretus* and *Rhinyptia* spp. (TREHAN, 1956; PAL, 1977) which emerge in large numbers with the onset of rains and feed on the foliage. *Holotrichia consanguinea* BLANCHARD. *Anomala bengalensis* BL. and *Adoretus* sp. were observed causing considerable damage to different ber cultivars at the Central Research Farm, CAZRI, Jodhpur. Studies on biology and control of these beetles have been carried out by various workers (KAUL *et al.*, 1966; JOSHI *et al*, 1969; RAI *et al.*, 1969; SHINDE & SHARMA, 1970; YADAVA & YADAVA, 1973). But practically no information is available about the relative perfor nance of different ber cultivars against the attack of the chafer beetles; and about the loss caused by different beetle species. Present investigations, therefore, were undertaken to evaluate ber cultivars for the loss caused by the chafer beetles and to study the damage potential of individual beetle species along with the insecticidal trial to find out effective insecticides to control the beetles.

MATERIALS AND METHODS

Observations were taken after the ber plants were attacked by the beetles following the rains. Six leaves at random were taken from each plant which served as one replicate. Three replications were taken for each of the fifteen cultivars. The leaf area consumed by the beetles was measured with a planimeter after drawing the damaged area on paper (Table 1).

For the relative loss caused by the three beetle species viz., *Holotrichia consanguinea* BLANCH., *Anomala bengalensis* BLANCH. and *Adoretus* sp., ber leaves were fed in a dark room to the individual beetle species starved for eight hours. Eight replications, each consisting of five beetles released in a wired cage (15 cm^3) with moist sand at the bottom were kept for each species. Sixty sq. cm of ber leaves of *Aliganj* and *Gola* cultivars (which, in the field were found to be the least and the most damaged cultivars respectively) were fed to the beetles of each species. Leaves were changed every 24 hours and moistened periodically to avoid drying. Dead beetles were replaced with previously starved beetles of the same species. Leaf area

consumed was drawn on paper and measured (Table 2).

Insecticidal trial was carried out in the laboratory on *Holotrichia* beetles using formulations and concentrations as shown in Table 3. Treated leaves were fed to the starved beetles kept in wired cages of 15 cm^3 with moist sand at the bottom. Control lot was fed with ber leaves treated with plain water. Observations were taken after every 24 hours for 72 hours. Mortality data were corrected and converted to arc sin values for statistical analysis.

RESULTS AND DISCUSSION

Data presented in Table 1 revealed that the cultivar *Aliganj* suffered the least damage in the field due to chafer beetles in terms of absolute leaf area consumption as well as per cent consumption. Whereas the maximum leaf area was consumed of *Gola* cultivar, the highest per cent leaf area loss was oberved in *Jogia* cultivar.

Table 2 shows the relative consumption under laboratory conditions, of leaves of cultivars *Aliganj* and *Gola* by the three beetle species. There was no significant difference in actual leaf consumption of *Aliganj* cultivar by the three species, nor in per cent leaf area consumption. In *Gola* cultivar also, difference in actual leaf consumption by all the three species was insignificant. However, *Adoretus* sp. caused significantly higher per cent leaf area loss than *Anomala* and *Holotrichia* species. TREHAN (1956) has also reported *Adoretus*

TABLE 1. Field damage by chafer beetles to different *ber* cultivars.

(Mean of 3 replications)

S. no.	Cultivar	Actual leaf area consumed (cm^2)	Per cent area consumption	
1.	Aliganj	1.67	17.70	(24.70)*
2.	Banarasi Pebandi	4.19	35.45	(36.49)
3.	Chhuhara	3.74	28.41	(32.15)
4.	Dandan	4.80	31.88	(34.15)
5.	Gola	6.63	42.62	(40.76)
6.	Ilaichi	5.85	30.10	(33.21)
7.	Jogia	5.24	48.30	(44.04)
8.	Kaithli Hissar	2.19	37.44	(37.77)
9.	Kali	4.94	34.67	(35.93)
10.	Mundia	3.85	32.20	(34.48)
11.	Rashmi	5.20	25.01	(29.99)
12.	Seb	4.91	39.43	(38.84)
13.	Sunahri Gola	3.50	24.90	(29.89)
14.	Thornless	4.30	32.66	(34.75)
15.	Umran	2.88	24.9	(29.67)
	SEm ±	0.75		2.53
	CD (p = 0.5)	2.18		7.32

* data in parentheses represent arc sin values of per cent consumption.

TABLE 2. Relative consumption of *ber* leaves by three species of chafer beetles

(Mean of 8 replications).

	Gola		Aliganj	
	Actual consumption (cm^2) per beetle	Per cent consumption	Actual consumption (cm^2) per beetle	Per cent consumption
1. *Adoretus* sp.	11.31	31.41	9.85	27.36
2. *Anomala bengalensis* BL.	7.01	20.06	6.92	20.02
3. *Holotrichia consanguinea* BL.	7.97	22.15	8.27	22.97
SEm±	**	1.98	**	**
C D (P = 0.05)	**	6.02	**	**

**F test insignificant.

TABLE 3. Relative efficacy of insecticides against adult *Holotrichia consanguinea* BLANCH.

(Mean of 3 replications).

S. no.	Treatment	Per cent concn. a i	Mean corrected mortality (%)	Mean arc sin values of corrected mortality
1.	Carbaryl (W P)	0.10	70.19	57.22
2.	Carbaryl (D)	10	63.06	52.59
3.	Dichlorvos (EC)	0.05	70.93	57.55
4.	Endosulfan (EC)	0.05	54.81	47.86
5.	Malathion (EC)	0.10	63.06	52.59
6.	Malathion (D)	5	61.39	51.99
7.	Methyl parathion(EC)	0.05	78.43	62.66
8.	Methyl parathion (D)	2	66.02	54.63
9.	Fenitrothion (EC)	0.05	81.30	64.66
10.	Phenthoate (EC)	0.05	59.35	50.41
11.	Quinalphos (EC)	0.05	77.22	62.11
12.	Control	—	—	—
	CD (p = 0.05)			11.43

to be the most serious beetle pest of ber in Punjab. Since all the three species under study attack ber foliage simultaneously, and there is almost no difference in the actual leaf consumption, no single beetle species can be considered to be a more important pest than the other two. However, their relative abundance can be a useful consideration to decide the status.

Insecticidal trial (Table 3) revealed the superiority of methyl parathion (EC), fenitrothion and quinalphos over other treatments, except dichlorvos, carbaryl (W. P.) and methyl parathion (dust) with

which these were found to be at par. All the insecticidal treatments were superior to control, but no statistically significant difference was observed in the beetle mortality due to dichlorvos, carbaryl (D), methylparathion (D), malathion (EC & D) phenthoate and endosulfan. These findings are in agreement with SHINDE & SHAKMA (1970) who reported methyl parathion, dichlorvos and malathion to be effective against *H. consanguinea* Bl. BINDRA & SINGH (1971) reported fenitrothion and carbaryl to be effective against the beetles of *Lachnosterna (Holotrichia) consanguinea*. BL. The results of present investigation are also supported by RAI *et al.* (1969) who reported higher mortality of *H. consanguinea* adults fed on foliage treated with methyl parathion, but a relatively low mortality of the beetles fed on endosulfan treated leaves. It may thus be inferred that methyl parathion, fenitrothion and quinalphos can be used for effective chafer beetle control.

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REPORTS AND NEW RECORDS

NEW RECORD OF AN EGG PARASITE, *GRYON* SP. ON *NEZARA VIRIDULA* (L.)

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(Received 10 May 1981)

A scelonid parasitoids belonging to *Gryon*, which seems to be the first record on *Nezara viridula* eggs in India is reported.

(Key words: scelonid, parasitoid, *Gryon* spp. *Nezara viridula* eggs)

The green bug, *Nezara viridula* (L.) (Hemiptera : Pentatomidae) is an important pest of pigeonpea, *Cajanus cajan* (L.) Millsp. in India (SINGH & SINGH, 1978). The bugs suck up the juice of unripe seeds from the green pods. Eggs are oviposited in masses in hexagonal shape mostly under the ventral surface of the leaves. The collection of egg masses were made for the study on parasitism during the month of January and February, 1979. The egg masses were caged in glass vials individually and were found to be parasitised by a scelonid parasitoid identified as *Gryon* sp. The parasitoid seems to be the first record on *N. viridula* eggs in India. An individual egg mass was observed to be completely parasitised. LAL *et al.*, (1975) and Anonymous (1978—1979) recorded *Gryon* sp. parasitising on the eggs of *Clavigralla gibbosa* (Spin.) also.

Thanks are due to the Director, British Museum, London for identifying the parasitoid.

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